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LEUKEMIA IN RADIOLOGISTS, TEN YEARS LATER

WITH A REVIEW OF THE PERTINENT EVIDENCE FOR RADIATION LEUKEMIA

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During the past decade there has been brought to light evidence tending to substantiate the hypothesis that ionizing radiation is one of the causes of leukemia. One of the manifestations is the increased incidence of leukemia in radiologists in the United States, to which we called attention in 1944 and 1950[39,40]. In leukemia we are dealing with a not too prevalent condition, so that, even with the demonstrated increased incidence, the number of cases occurring in radiologists is relatively small (14 from 1929 to 1948 inclusive[40]). It was, therefore, felt that it was advisable to extend this study for a longer period of time to include a greater number of cases in order to increase the validity of the previous observations. Furthermore, a trend toward a different incidence might be disclosed. And finally, because a recent report (Court-Brown and Doll[11]) from England of a study made of the incidence of leukemia in British radiologists does not bear out the findings in the United States, it was deemed important to extend our knowledge of this phase of the problem. Court-Brown and Doll made a study of 1377 living and dead male radiologists in Britain for the 60-year period from 1897 to 1956. They found only 3 deaths from leukemia, 2 of which occurred in a group of radiologists who became members of a radiological society prior to 1921 (the time before which the authors feel that the need for adequate protection by the radiologists was not generally appreciated), whereas the expected number for this sex, age, and social class distribution was 0.3 to 0.5 death. In the post-1921 radiologists there was only one death from leukemia reported, and the expected number was 0.7 to 0.9 death.

The present report is concerned with an extension of our previous statistics to cover the decade from 1949 to 1958 inclusive. The same methods were employed as in the previous reports. The total number of deceased radiologists during this period was gathered from the records of the American College

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of Radiology, the American Roentgen Ray Society, and the Radiological Society of North America. In the previous studies it had been felt that for practical purposes this can be regarded as a fairly complete listing, despite the fact that there may be a rare practicing radiologist who may not be a member of one of these Societies or who may have dropped from membership because of the economic pressure of retirement or prolonged illness. During the course of the present investigation it became apparent that the list of deceased radiologists thus compiled was not complete, and so a close personal scrutiny of all of the obituary notices published in the Journal of the American Medical Association during this 10-year period was made, and this uncovered an additional 102 deceased radiologists. Included in the group of deceased radiologists were all those listed as certified by the American Board of Radiology, as members of the American College of Radiology, as practicing radiologists (or roentgenologists), as members of a hospital radiology department, as having an academic position in radiology, or as being members of the American Roentgen Ray Society. If a physician was listed as being a member of the Radiological Society of North America, he was not regarded as a radiologist for our purposes unless there was some supporting item from the preceding list, since numerous surgeons, internists, dermatologists, gynecologists, and others are members of this Society. We feel that this exclusion is entirely justified, and that we would be much farther from the truth to include the 26 cases found with this Society listing as their sole professional identification than to exclude them. Six additional cases were excluded because of very doubtful evidence, although 2 or 3 with somewhat doubtful evidence were included in the tabulated cases. As before, only residents of the United States were included.

We are, therefore, reporting two sets of figures: those obtained by following the methods pursued in the previous studies, to serve as comparative figures, and the more complete figures obtained by the procedure indicated in the preceding paragraph. Following the original procedure, notices of the deaths of 302 radiologists were disclosed during the 1949 to 1958 decade, in which the cause of death could be determined in 279. In the additional 102 cases the cause of death could be determined in 82, making a total of 404 deceased radiologists in which the cause of death was known in 361. It is to be noted that the 279 figure for this decade is almost as great as the 299 figure for the combined preceding two decades (March[40]). In the 279 deaths, there were 9 cases of leukemia (3.23%), as indicated in Table 1. In the 82 additional deaths there were 2 cases of leukemia, making a total of 11 cases in 361 (3.05%).

The incidence of leukemia deaths in radiologists (3.23%) during the decade under consideration is somewhat less than the comparable figures found for the preceding 20 years (4.68%), but is still considerably more than the incidence in non-radiological physicians (0.51%)[40], the latter figure being taken from the previous studies of the preceding two decades, since the information in the Journal of the American Medical Association during the past decade is too incomplete for figures to be derived directly for non-radiological physicians. The frequency has thus decreased from about nine times the incidence in non-radiological physicians to about four or five times. This still appears statistically significant.

It may be mentioned that 4 deaths of radiologists were listed as aplastic

TABLE 1.—RADIOLOGISTS DYING OF LEUKEMIA (1949 TO 1958 INCLUSIVE)

Initials	*Date of Death*	*Type of Leukemia Recorded*	*Age*	*Location*
R.H.L.	7/31/50	Acute	72	Charlotte, N.C.
W.E.P.	8/19/51	Myelogenous	48	Morristown, N.J.
C.C.T.	11/15/51	Lymphatic	69	Greene, Me.
J.M.B.	11/26/52	Myelogenous	74	Middleboro, Mass.
R.F.M.	5/ 9/53	Leukemia	50	Chicago, Ill.
*R.T.P.	8/27/54	Leukemia	63	Springfield, Mass.
B.V.L.	7/25/55	Leukemia	55	San Francisco, Cal.
W.A.R.	8/13/55	Acute	43	Augusta, Me.
G.E.P.	1/19/57	Leukemia	83	Philadelphia, Pa.
*J.G.H.	2/18/57	Leukemia	49	San Francisco, Cal.
D.J.R.	3/12/57	Acute Lymphatic	64	Hartford, Conn.

*Cases uncovered by present more complete procedure.

anemia. It is not known whether any of these might in reality have been forms of leukemia, since Court-Brown and others feel that some cases of leukemia probably are listed as aplastic anemia; 4 out of 6 cases of leukemia following either therapeutic or heavy diagnostic Roentgen-ray exposure reported by Moloney[45] simulated aplastic anemia at the onset, and the diagnosis was determined only after bone marrow aspiration or biopsy. In the case of one radiologist, L.F.T., of Marshalltown, Iowa, who died in 1952, the given cause of death was chronic radiation syndrome. Further investigation revealed that necropsy disclosed a marked preponderance of plasma cells and plasmoblasts in the bone marrow and lymph nodes. The pathologist at the University of Iowa Hospital was unwilling to classify this as a leukemia, but felt that this was a peculiar reticular cell response to radiation. The internist regards this case as a reticuloendothelial dysplasia with plasma cell leukemoid response, a borderline category with many aspects of leukemia. However, this case was not included among the cases of leukemia.

After the data for this report had been assembled and near the end of a review of literature, a recent article (Cronkite, Moloney and Bond[13]) was encountered within which a brief mention was made of a survey similar to the present one being reported, but covering only 9 of the 10 years included in this paper. The present data cover a longer period and appear to be more complete (19 additional radiologists' deaths with known diagnoses in that 9-year period).

It has been shown that the recorded incidence of leukemia in the general population has been increasing over the past several decades (Court-Brown and Doll[10], Lea and Abbatt[34], Sacks and Seeman[51], Schwartz and Upton[53]). It is difficult to assess how much of a factor better diagnoses may have played or whether increased exposure to ionizing radiation may not have been a factor. A 73% increase in white male death rates from leukemia above the age of 25 occurred from the 1939-1941 period to the 1949-1951 period (Lewis[35]). In the State of Illinois the case rate of leukemia and aleukemia increased from 5.1 for the 1941-1945 period to 7 for the 1953-1957 period[37]. One cannot calculate the leukemia incidence in physicians in the last few years because of the incompleteness of

the reported causes of death in the Journal of the American Medical Association, but it also has probably increased, though probably not to the same degree, since better diagnosis would not be as much of a factor. There would appear, therefore, to have been a moderate decrease in the incidence of leukemia in radiologists in the past decade as compared with the preceding 20 years. One hopes that this is a reflection of better protection against exposure by the radiologist since the earlier days of this specialty.

That ionizing radiation can induce leukemia has been demonstrated in laboratory animals many times (Furth[15], Furth and Lorenz[17], Gross *et al.*[22], Kjeldsberg[31], March[40], Mole[42], Upton[66]). Five additional groups of statistics have appeared demonstrating this effect in man with varying degrees of persuasiveness. These concern the atomic bomb casualties, patients with rheumatic spondylitis treated with Roentgen-ray, patients whose thymic areas were irradiated, children who had been irradiated *in utero*, and patients treated with radioactive iodine for hyperthyroidism and thyroid malignancy.

The atomic bombing of Hiroshima and Nagasaki in August, 1945, resulted in a total body irradiation of a large number of persons by a single exposure to gamma rays and neutrons. Carefully documented follow-up studies by members of the Atomic Bomb Casualty Commission at various intervals have revealed that by December 31, 1957, there were 122 well authenticated cases of leukemia in the exposed population of Hiroshima (Heyssel *et al.*[24]), 96 being in individuals exposed less than 2,000 meters from the hypocenter (the point on the ground directly beneath the bomb blast). It is well known that generally the survivors who had been within a 1,000 meters of the hypocenter had been heavily irradiated, while the group of survivors beyond 2,000 meters had received negligible irradiation (Heyssel *et al.*[24], Moloney and Kastenbaum[46]). Heyssel *et al.* found that the incidence of leukemia in the latter group was no greater than that recently estimated for Japan as a whole (29 as against 24 cases per million per year). By contrast, the incidence in the group within 1,000 meters was 50 to 60 times the incidence beyond 2,000 meters. A single massive exposure to irradiation is, therefore, leukemogenic in humans just as it has shown to be in mice (Furth and Upton[18]). The figures from Nagasaki are similar to those from Hiroshima (Moloney[43]). Of interest is the time relationship of the uncovering of these leukemia cases. For 2 years after the bombing no cases of leukemia were reported. Following this, cases began to appear with increasing frequency, reaching a peak some 5 to 7 years after the bombing and subsequently diminishing, but the normal incidence of spontaneous leukemia had not yet been reached after 13 years (Heyssel *et al.*[24]). The initial "latent" period may in part have been due to the general disruption of medical facilities and record keeping, but similar latent periods have been observed in other situations where ionizing radiation has been etiologically related to leukemia, as in experiments with mice (Furth and Furth[16]). Many feel that a latent period of at least a year be required to possibly associate a case of leukemia with preceding irradiation (Court-Brown and Doll[10], Moloney[44]), although a very occasional case with a shorter latent period may seem to merit consideration (Graham[20]). The length of the latent period may be many years (Heyssel *et al.*[24]).

Leukemia due to atomic radiation is similar clinically and pathologically to spontaneous leukemia in the Japanese

(Hollingsworth[26], Moloney[43]). Myelogenous leukemia was much the commonest, and acute leukemia occurred, but chronic lymphatic leukemia was rare (Kimball[30]). The latter type is uncommon in Japan normally.

There have been several reports of the increased incidence of leukemia in patients with rheumatoid spondylitis treated by Roentgen-ray therapy. Some concern isolated cases or groups of cases, without statistical reference (Graham[20], Graham[21], van Swaay[67]). In a group of 31 cases of leukemia reported from Norway, 8 had had Roentgen therapy to the spine for arthrosis-spondylosis (Voll and Tveit[69]). In the past few years 3 statistical reports have appeared. In Britain, Abbatt and Lea[2] in a series of 1627 men treated by radiotherapy for ankylosing spondylitis found 7 cases of leukemia deaths, whereas only 0.33 such death could have been expected. In a smaller (399 men) group of controls no leukemia deaths occurred, and 0.17 would have been expected, a very good agreement. Court-Brown and Doll[10] conducted an intensive investigation of 13,352 patients treated with Roentgen-ray for ankylosing spondylitis at 81 British radiotherapy centers from 1935 to 1954. There were 353 deaths in this group, of which 28 were due to leukemia. The expected incidence would have been 2.9. In this country Silberberg, Frohman and Duff[56] found that 5 patients developed leukemia (from 2½ to 10 years following the initial doses) in 496 patients treated from 1938 to 1954, whereas the expected incidence of leukemia in a population of this age and sex distribution would have been 0.29, an increase of 17.3 times.

The theoretical possibility that there is an association of leukemia with rheumatoid spondylitis in the absence of therapeutic radiation was investigated by Abbatt and Lea[3] in 679 cases of leukemia, but their statistics concern rheumatic disease in general, and while their studies purport to show some association between unirradiated rheumatic disease and leukemia, the category "rheumatic disease" appears too broad to allow any conclusions to be drawn with reference to rheumatoid spondylitis. They state that they believe that radiation plays a major part in the production of leukemia in ankylosing spondylitis, but warn against reliance on such cases in investigations of dose-response effects in radiation-induced leukemia.

A fifth approach to the relationship between ionizing radiation and leukemia has been made through studies of the incidence of leukemia in children who had been exposed to therapeutic doses of Roentgen-ray, particularly to the region of the thymus. Simpson, Hempelmann and Fuller[60] reported 7 cases of leukemia in 1400 infants who had had their thymus treated, whereas the expected would have been 0.6 case. In a control group of 1795 siblings there were no leukemia cases. In subsequent reports (Simpson[58], Simpson and Hempelmann[59]), this study has been extended to include 2,074 traced treated infants, among whom there developed 9 cases of leukemia, whereas the expected incidence would be 0.9 case. In the control group of untreated siblings no leukemia cases were observed, whereas 1.1 cases would have been expected.

In a study in Monroe County, New York, of 6,473 children under the age of 20 who had received irradiation for diseases other than cancer since 1930 (about 1 in 40 born since then) Murray, Heckel and Hempelmann[47] found 7 cases of leukemia in 3,224 such patients treated over the chest, and one in 1,073 cases treated about the head and neck. In a similar population that would not have been irradi-

ated, only 2 cases of leukemia would have been expected. In addition, a retrospective study was done on 65 of the 75 children dying of leukemia between 1940 and 1957 to determine prenatal and postnatal exposure. The 8 cases cited represent 12.3% of the total. This percentage is 6 to 10 times greater than the percentage of controls receiving "deep" Roentgen-ray therapy. Another retrospective study was done by Polhemus and Koch[49] on a group of 251 children with leukemia, mostly acute lymphogenous, seen between 1950 and 1957, with a similar group of controls matched for age, location (California), and other details. Twelve (4.8%) of the leukemic children had received previous thymic radiation or its equivalent. Only two (0.8%) of the controls had been so treated. This six-fold increase he considered highly significant. He also found an increased frequency of fluoroscopy and diagnostic roentgenography in the leukemics as compared with the controls, but not at a highly significant level.

Stewart, Webb, and Hewitt[64] studied a group of 619 deaths from leukemia in children under 10 occurring from 1953 to 1955 in England and Wales, together with a control group of similar size matched for age, sex, and locality. Ninety of the leukemia cases had had diagnostic Roentgen-ray exposures while 80 of the controls were found to have had such exposures. Five of the leukemia cases had had therapeutic radiation exposure and one of the controls. However, all of these therapy cases were instances of superficial therapy in girls for naevi, none being cases of thymic irradiation. While the authors state that the frequency of postnatal Roentgen-ray exposures was significantly higher than in the controls, the figures cited do not seem impressive; they admit that their figures indicate that the effect of postnatal Roentgen-ray exposure is much weaker than the effect of prenatal Roentgen-ray exposure, of which more will be said in a subsequent class of evidence to be considered.

In a preliminary study in this country by Snegireff[61] of 148 children whose thymic areas had been irradiated prior to 1946, and 185 controls, no cases of leukemia were found in either group. The small size of these samples does not permit any statistical validity. He quotes an unpublished report by Farber that of 856 cases of acute leukemia recorded between 1947 and 1958 at the Tumor Clinic of the Children's Cancer Research Foundation in Boston only 11 had Roentgen therapy in infancy, of which 5 were for enlarged thymus, a rate of 0.0058. Latourette and Hodges[33] reviewed 867 patients who had had thymic irradiation from 1932 to 1951 and found one case of leukemia, within the expected case rate for the size and age distribution of this sample. A similar study by Conti *et al.*[7] on a group of 1564 children who had received *routine* Roentgen-ray treatment to the thymus gland in Pittsburgh between 1938 and 1946 disclosed no cases of leukemia, although one case would have been expected in a group of this size and age distribution. In the control group of 2,923 siblings of these children, one case of leukemia occurred. Conti *et al.* suggest that the difference between their findings and those in which an increased incidence of leukemia was found could perhaps be explained by the small size of the portal used in their group (4 × 4 cm.). In a recent prospective study in Cincinnati, Saenger *et al.*[52] found in 1,644 children who had received Roentgen therapy to the chest, head, or neck only one case of leukemia, whereas in 3,777 unirradiated sibling controls there were 3 cases of leukemia. The incidence of leukemia was, therefore, approximately the same in both groups.

Several of the authors have pointed

out that one of the difficulties with this problem was the lack of a sizable control group of children with enlarged thymuses who had not been irradiated, in order to exclude an association between enlarged thymus itself and leukemia. Numerous variables, such as the dosage given in different groups considered, the size of the portals used, and the age at which the therapy was administered may in part contribute to the difference in the findings of the various studies.

It might at this point be noted that a recent study (Simon, Brucer and Hayes[57]) of the incidence of leukemia in 71,582 women treated by radiotherapy for carcinoma of the cervix showed no significant increase in such incidence (16 cases of leukemia developing after the therapy) over the expected one, even though the volume of bone marrow irradiated by such therapy is not inconsiderable.

The association of leukemia with the use of radioactive iodine is of interest in the present discussion. While no statistical studies have as yet been made, several case reports describing this association have appeared. These have been noted after the relatively heavier doses in cases of thyroid carcinoma (Blom, Querido and Leeksma[4], Jelliffe and Jones[27], Seidlin, Siegel and Yalow[54,55]), as well as after the smaller doses administered in thyrotoxicosis (Abbatt, Farran and Greene[1], Burns, Vickers and Lowney[6], Vetter and Höfer[68]). Ten cases of the latter association have now been reported, but as pointed out by Werner and Quimby[70], as well as Vetter and Höfer[68], the number of patients treated with I_{131} is now of sufficient magnitude that the occurrence of these relatively few cases could be a chance association alone of spontaneous leukemia in patients with thyrotoxicosis. The latter point out, however, a noteworthy time relationship in that there was a period of 18 months between the administration of the I_{131} and the onset of acute leukemia in 5 of these reported cases, and they feel that this may be an indication of a causal rather than a casual relationship. Seidlin *et al.*[55] reported 2 cases of acute myeloid leukemia in 16 cases treated by I_{131} for metastatic thyroid cancer. Solomon and Rubenfield[62] report the first case of chronic lymphatic leukemia associated with I_{131} given for hyperthyroidism and feel that it is merely coincidental. Pochin[48], at this writing, speaks of 10 additional unpublished cases of leukemia occurring in patients treated by radioiodine for thyrotoxicosis, making a total of 18, 3 in the United Kingdom, 10 in the United States, 4 in Canada, and 1 in Austria. He calculates that 60,000 thyrotoxic patients have been treated with radioiodine in these four countries alone, with an estimated total of over 220,000 patient-years since treatment until mid-1960. Application of natural leukemia rates appropriate to the sex, age, and nation of these patients suggests that approximately 21 cases of "spontaneous" leukemia would have been expected to occur among them, and so the present known incidence of leukemia following radioiodine therapy of thyrotoxicosis gives no indication that this treatment is inducing leukemia. He further indicates that the intervals between radioiodine treatment and the diagnosis of leukemia in the known cases are consistent with a chance variation.

Between the time of publication of the preliminary communication of Stewart *et al.*[63] in 1956 and their final report[64] in 1958 on the relationship between antenatal irradiation and the subsequent development of leukemia in children, considerable discussion (Rabinowitch[50]) was provoked as to the validity of the inferences drawn.

In the initial paper they reported that in 269 cases of leukemia (approximately 1/3 of the cases of children under 10 dying of leukemia in the triennium 1953-1955 in England and Wales) 42 of the mothers gave a history of some pre-partum diagnostic Roentgen-ray procedure to the abdomen, while in a corresponding number of case-matched controls, only 24 mothers gave such a history. A similar disparity was found in regard to other malignant diseases killing children in this triennium, but since our concern in this paper is with leukemia alone, nothing further shall be said of this phase of their work, just as the association with other malignancy was omitted in the section dealing with irradiation of the thymic area. In their final report, 619 leukemia cases (and 680 other cancers) are considered, but unfortunately no distinction was made between the leukemia cases and the cases of other malignancies in the presentation that 178 mothers of the combined groups (leukemia and other malignancies) had reported abdominal roentgenologic examinations during the pregnancy whereas only 93 of the controls had reported such examinations. It is interesting that in a breakdown of the time during pregnancy that these examinations occurred, a vast majority occurred after the fifth month, yet the authors state that "the variations in the second half of pregnancy (*i.e.* between case and control figures) all lie within the accepted range of chance variation." Nevertheless, in their summary they state that the frequency of prenatal irradiation was "significantly higher among the dead children than among the live children," but they conclude that "foetal irradiation does not account for the recent increase in childhood malignancies, but the finding of the case excess for this event does underline the need to use minimal doses for essential medical x-ray examinations and treatment."

In a similar study Ford, Paterson and Treuting[14] investigated 78 leukemia deaths in children under 10 occurring in Louisiana during 1951-1955. Their control group consisted of 306 children (matched for age, sex, and race) who had died of other causes. The controls were, therefore, of a different category from Stewart's. She found that 26.9% of the leukemics had antenatal exposure, and 18.3% of the controls, and she concludes: "These differences, while suggestive, do not justify a conclusion that the relative frequencies of exposure to antenatal irradiation differ in the populations represented by the sample data. It is the similarity of the results in this study and that of Stewart that is of primary interest."

From Norway, Kjeldsberg[81] reported on 55 leukemic children together with 55 controls. He found five (9%) of the leukemic cases had had antenatal irradiation and eight (14.5%) of the controls and, therefore, did not corroborate Stewart's or Ford's findings. At the present writing Court-Brown, Doll and Hill[12] report on 39,166 children born to women who had abdominal roentgenologic examinations in 8 metropolitan hospitals in 1945-1946 and found 9 deaths from leukemia, whereas 10.5 deaths would have been expected. Their findings again did not confirm those of Stewart and Ford. In the study of Murray, Heckel and Hempelmann[47] there was no indication of excess irradiation of the leukemic group *in utero* as compared with the controls. In fact, some of the control groups had slightly higher pelvimetric and other abdominal Roentgen-ray examination percentages than the leukemic group, but because the cases involved were few, valid testing was impossible. In 40 cases of leukemia in children seen

in one clinic in Budapest from 1953 to 1957, Gefferth[19] found that the percentage of antenatal exposure was somewhat greater than in 346 controls (children being seen at the clinic for some other condition), but he does not consider this significant. Train[65] found one case of leukemia in 3,043 mothers who had Roentgen-ray pelvimetry at a maternity hospital from 1945 to 1956. There was no control group. This finding is not significant of any increased incidence. In Cornwall, Wood[71] found in two institutions during an 8- and 11-year period, respectively, a total of 713 pregnant women who had either abdominal Roentgen-ray examinations or Roentgen pelvimetries, and no leukemic child was found resulting from any of these pregnancies. Of the 20 cases of leukemia in children under 10 that occurred in the years investigated, the mother of only one had had an abdominal Roentgen-ray during pregnancy. In 1958, McMahon[41] reported a 1% sampling (about 2500) of all the children born alive in 11 selected maternity hospitals in New York City between 1947 and 1954, and it was found that the percentage of their mothers who had received pre-partum diagnostic Roentgen-ray examination (7.3%) corresponded exactly to the percentage of pre-partum irradiated mothers of children under 10 subsequently dying of leukemia and other cancer during this period who had been born in these hospitals. Kaplan[28], in 1958, reported his findings in children dying of acute leukemia in California in 1955 and 1956. Using two sets of controls, the child's closest sibling and the child's habitual playmate, and investigating the prenatal radiation history in all three groups, he found equivocal results depending on which set of controls one used for comparison: abdominal Roentgen-ray exposure was significantly more frequent in the pregnancy of the leukemic child than during the sibling pregnancy but not compared to the pregnancy of his unrelated playmate. Kaplan concludes that the evidence regarding the leukemogenic hazard of pelvimetry is still unconvincing. In a study at a British maternity hospital, Lewis[36] was able to find 8 cases of children under 10 years dying of leukemia from 1953-1958 that developed among 45,195 live births between 1943 and 1958, whereas 6.6 cases would have been expected. Seven of these received no prenatal irradiation, an incidence of 1:1808 among the 12,657 non-irradiated live births for a 6-year period. Only one received prenatal irradiation, an incidence of 1:4291 among the 4,291 pre-partum irradiated live births during a 6-year period.

A report by Manning and Carroll[38] that in 188 cases of leukemia in children under 14 she found 36 mothers (19.5%) had received radiation therapy prior to or during pregnancy compared with 11.9% of control mothers does not merit consideration in this respect because of the irrelevant lumping of "prior to" with "during" and because these figures are based on unverified answers to a questionnaire. Moreover, in practically all of these cases the therapy was to a site remote from the abdomen, and the majority was dermatologic.

It is generally agreed that there is a causal relationship between ionizing radiation and some cases of leukemia. The nature of this relationship is under continued investigation, particularly in regard to the dose-response aspect. The study of Court-Brown and Doll[10] on ankylosing spondylitis was significant from this standpoint since the doses were known. They feel that following all but the highest doses of radiation there is a linear relationship, that is, the incidence of leukemia is

directly proportional to the dose received by the bone marrow (Court-Brown[8,9], Court-Brown and Doll[10]). However, in the introduction to the report, the British Medical Research Council[10] states that "It is possible to derive more than one type of dose-response relationship from the data." The most controversial aspect, however, is whether this linear relationship holds for very small doses or whether there is a threshold below which leukemia is not produced. Court-Brown[9] feels that a non-threshold effect; that is, a simple proportional relationship holding for even the lowest doses, is a reasonable working hypothesis. On this the Council says with commendable conservatism, "One cannot either extrapolate the data to obtain an indication of the possible leukemogenic effects of small doses of radiation or obtain it directly since few of the patients had received less than a mean dose of 250 r to the bone marrow." It is worthy of note that the doses and portal sizes used in these cases are generally larger than is customary in this country. The findings of Court-Brown and Doll and of Abbatt and Lea have been discussed and evaluated by a number of writers, including Hempelmann[23].

The data (Heyssel *et al.*[24], Lewis[35]) from the atomic bombs of Hiroshima and Nagasaki have been accepted by most to show a linear dose-response relationship since there was an increasing number of leukemia cases as one approached the hypocenter, and the dose received, of course, varied crudely with the distance. This held for distances corresponding to dosages above 50 or 75 r (Heyssel and Meneely[25], Hollingsworth[26]). Here also the data provide no evidence for or against a threshold dose below 75 r (Heyssel and Meneely[25]). Brues[5], however, states that the present data from the atomic bombings "are at least as compatible with a physiological mechanism linking leukemogenesis with initial severe hematopoietic damage, or with some other non-linear relation, as they are with one assuming an effect that is strictly linear with dose." Brues likewise feels that the actual data of Court-Brown and Doll's work with ankylosing spondylitis indicate that a working hypothesis of linearity is a less probable one than one that assumes a threshold or non-linear relation. Since a consideration of threshold dose by an extrapolation of high dose data is fraught with difficulties and uncertainties, one must examine the only direct evidence in man that small doses of radiation delivered in a relatively short time are leukemogenic, namely, the studies of Stewart, Webb and Hewitt[64] and Ford, Paterson and Treuting[14] on the influence of antenatal Roentgen-rays. As has been seen above, this evidence is not strongly convincing.

A widely quoted paper by Lewis[35] analyzes the reported data on the atomic bombings, Court-Brown and Doll's data on ankylosing spondylitis, Simpson's data on thymic irradiation, and the data on leukemia in radiologists. He accepts the linear dose-response relationship of the bombings and the spondylitis cases, and after making certain assumptions, he derives from each of these four sources a probability of developing leukemia per individual per rad per year that approximate each other to a fair degree. Brues[5], however, points out certain flaws in this approach and concludes that present data on human leukemogenesis fail to indicate a linear relation between dose and effect. A linear hypothesis cannot be ruled out statistically because of the paucity of the data, but he states that it is less probable than a non-linear or threshold relation. Moreover, he makes the

further point that other instances in which carcinogenic agents have been examined from the standpoint of dose and dose-rate relations show many clear instances where the relation is non-linear and none in which linearity is unquestionably demonstrated. Kimball[30] concludes from a strict statistical viewpoint that Lewis's presentation neither supports nor disproves the hypothesis of linearity. Hempelmann also calls attention to corrections that should be made in Lewis's calculations[23].

Summary. 1. The incidence of death from leukemia in radiologists in the United States for the decade 1949 to 1958 is about four or five times as great as in non-radiological physicians.

2. This is approximately one-half the differential that existed in the preceding two decades.

3. It is anticipated that with awareness of the problem and the taking of adequate protection by radiologists, this differential will tend to diminish still further, but will not entirely disappear.

4. There is no doubt concerning the causal relationship of ionizing radiation with some cases of leukemia. A review of the major avenues of evidence for this relationship has been presented. The most positive evidence comes from the experimental laboratory, from the atomic bombing data, from the studies in radiologists, and from Roentgen therapy in ankylosing spondylitis. Quite suggestive evidence comes from irradiation of the infantile thymus. Quite doubtful evidence has been associated with the therapeutic use of radioactive iodine and with antenatal fetal diagnostic irradiation.

5. The majority of informed opinion holds that the evidence points toward a linear dose-response relationship in radiation leukemogenesis, but there is good authority also for the concept of a non-linear relationship. As to whether there is a linear relationship for small doses or whether there is a threshold dose, this remains an unsettled problem.

REFERENCES

1. **Abbatt, J. D., Farran, H. E. A.,** and **Greene, R.:** Lancet, **1**, 782, 1956.
2. **Abbatt, J. D.,** and **Lea, A. J.:** Ibid., **2**, 1317, 1956.
3. Iidem: Ibid., **2**, 880, 1958.
4. **Blom, P. S., Querido, A.,** and **Leeksma, C. H. W.:** Brit. J. Radiol., **28**, 165, 1955.
5. **Brues, A. M.:** Science, **128**, 693, 1958.
6. **Burns, T. W., Vickers, R.,** and **Lowney, J. F.:** Arch. Int. Med., **106**, 97, 1960.
7. **Conti, E. A., Patton, G. D., Conti, J. E.,** and **Hempelmann, L. H.:** Radiology, **74**, 386, 1960.
8. **Court-Brown, W. M.:** Brit. Med. Bull., **14**, 168, 1958.
9. Idem: J. Chr. Dis., **8**, 113, 1958.
10. **Court-Brown, W. M.,** and **Doll, R.:** Leukemia and aplastic anemia in patients irradiated for ankylosing spondylitis. Medical Reserch Council Special Report 295, Her Majesty's Stationery Office, London, 1957.
11. Iidem: Brit. Med. J., **2**, 181, 1958.
12. **Court-Brown, W. M., Doll, R.,** and **Hill, A. B.:** Ibid., **2**, 1539, 1960.
13. **Cronkite, E. P., Moloney, W. C.,** and **Bond, V. P.:** Am. J. Med., **28**, 673, 1960.
14. **Ford, D. D., Paterson, J. C. S.,** and **Treuting, W. L.:** J. Nat. Cancer Inst., **22**, 1093, 1959.
15. **Furth, J.:** Radiation neoplasia. Proceedings of Third National Cancer Conference, June, 1956. Philadelphia: J. B. Lippincott Co., 1956.
16. **Furth, J.,** and **Furth, O. B.:** Am. J. Cancer, **28**, 54, 1936.
17. **Furth, J.,** and **Lorenz, E.:** Radiation Biology, Vol. I, part 2, Chapter 18. (Edited by A. Hollaender.) National Research Council, New York: McGraw-Hill Book Co., 1954.
18. **Furth, J.,** and **Upton, A. C.:** Acta radiol. (supp. 116), p. 469, 1954.

19. **Gefferth, K.:** Strahlentherapie, **108**, 107, 1959.
20. **Graham, D. C.:** Arch. Int. Med., **105**, 51, 1960.
21. **Graham, W.:** Ann. Rheumat. Dis., **7**, 245, 1958 (in Discussion).
22. **Gross, L., Roswit, B., Mada, E. R., Dreyfuss, V.,** and **Moore, L. A.:** Cancer Res., **19**, 316, 1959.
23. **Hempelmann, L. H.:** Ibid., **20**, 18, 1960.
24. **Heyssel, R. M., Brill, A. B., Woodbury, L. A., Nishimura, E. T., Ghose, T., Hoshimo, T., Yamasaki, M.:** Blood, **15**, 313, 1960.
25. **Heyssel, R. M.,** and **Meneely, G. R.:** Am. J. Med., **29**, 187, 1960.
26. **Hollingsworth, J. W.:** New England J. Med., **263**, 481, 1960.
27. **Jelliffe, A. M.,** and **Jones, K. M.:** Clin. Radiol., **11**, 134, 1960.
28. **Kaplan, H. S.:** Am. J. Roentgenol., Rad. Therapy and Nuclear Med., **80**, 696, 1958.
29. **Kaplan, H. S.:** Radiation-induced leukemia in mice: a progress report. Radiation Biology and Cancer, Austin: University of Texas Press, p. 289, 1959.
30. **Kimball, A. W.:** J. Nat. Cancer Inst., **21**, 383, 1958.
31. **Kjeldsberg, H.:** Tidsskr. norske laegefor., **77**, 1052, 1957.
32. **Lange, R. D., Moloney, W. C.,** and **Yamawaki, T.:** Blood, **9**, 574, 1954.
33. **Latourette, H. B.,** and **Hodges, F. J.:** Am. J. Roentgenol., Rad. Therapy & Nuclear Med., **82**, 667, 1959.
34. **Lea, A. J.,** and **Abbatt, J. D.:** Lancet, **2**, 231, 1958.
35. **Lewis, E. B.:** Science, **125**, 965, 1957.
36. **Lewis, T. L. T.:** Brit. Med. J., **2**, 1551, 1960.
37. Malignant Neoplasm Deaths, Illinois Residents. Department of Public Health, State of Illinois, Springfield, May, 1959.
38. **Manning, M. D.,** and **Carroll, B. E.:** J. Nat. Cancer Inst., **19**, 1087, 1957.
39. **March, H. C.:** Radiology, **43**, 275, 1944.
40. Idem: Am. J. Med. Sci., **220**, 282, 1950.
41. **McMahon, B.:** Paper read to the American Public Health Association, Dec., 1958.
42. **Mole, R. H.:** Brit. Med. Bull., **14**, 174, 1958.
43. **Moloney, W. C.:** New England J. Med., **253**, 88, 1955.
44. Idem: Induction of leukemia in man by radiation. Radiation Biology and Cancer, Austin: University of Texas Press, p. 310, 1959.
45. Idem: Blood, **14**, 1137, 1959.
46. **Moloney, W. C.,** and **Kastenbaum, M. E.:** Science, **121**, 308, 1955.
47. **Murray, R., Heckel, P.,** and **Hempelmann, L. H.:** New England J. Med., **261**, 585, 1959.
48. **Pochin, E. E.:** Brit. Med. J., **2**, 1545, 1960.
49. **Polhemus, R. W.,** and **Koch, R.:** Pediatrics, **23**, 453, 1959.
50. **Rabinowitch, J.:** Lancet, **2**, 1261, 1956.
51. **Sacks, M. S.,** and **Seeman, I.:** Blood, **2**, 1, 1947.
52. **Saenger, E. L., Silverman, F. N., Sterling, T. D.,** and **Turner, M. E.:** Radiology, **74**, 889, 1960.
53. **Schwartz, E. C.,** and **Upton, A. C.:** Blood, **13**, 845, 1958.
54. **Seidlin, S. M., Yalow, A.,** and **Siegel, E.:** Radiology, **63**, 797, 1954.
55. **Seidlin, S. M., Siegel, E., Yalow, A. A.,** and **Melamed, S.:** Science, **123**, 800, 1956.
56. **Silberberg, D. H., Frohman, L. A.,** and **Duff, I. F.:** Arthritis and Rheumatism, **3**, 64, 1960.
57. **Simon, N., Brucer, M.,** and **Hayes, R.:** Radiology, **74**, 905, 1960.
58. **Simpson, C. L.:** Radiation Biology and Cancer. Austin: Univ. of Texas Press, p. 336, 1959.
59. **Simpson, C. L.,** and **Hempelmann, L. H.:** Cancer, **10**, 42, 1957.
60. **Simpson, C. L., Hempelmann, L. H.,** and **Fuller, L. M.:** Radiology, **64**, 840, 1955.
61. **Snegireff, L. S.:** Ibid., **72**, 508, 1959.
62. **Solomon, S.,** and **Rubenfield, S.:** Arch. Int. Med., **106**, 428, 1960.
63. **Stewart, A., Webb, J., Giles, D.,** and **Hewitt, D.:** Lancet, **2**, 447, 1956.
64. **Stewart, A., Webb, J.,** and **Hewitt, D.:** Brit. Med. J., **1**, 1495, 1958.
65. **Train, T. S. R.:** J. Obst. & Gyn. Brit. Emp., **67**, 273, 1960.
66. **Upton, A.C.:** Studies on the mechanism of leukemogenesis by ionizing radiation. U.S. School of Aviation Medicine. Project Report 59-17, Jan., 1959.
67. **van Swaay, H.:** Lancet, **2**, 225, 1955.

68. **Vetter, H.,** and **Höfer, R.:** Brit. J. Radiol., **32,** 263, 1959.
69. **Voll, A.,** and **Tveit, J.:** Nordisk Medicin, **58,** 1114, 1957.
70. **Werner, S. C.,** and **Quimby, E. H.:** J. Am. Med. Assn., **165,** 1558, 1957.
71. **Wood, E. E.:** Brit. Med. J., **1,** 1760, 1960.

SUMMARIO IN INTERLINGUA

Leucemia in Radiologos. Dece Annos plus Tarde Con un Revista del Datos Pertinente con Respecto a Leucemia de Radiation

1. Le incidentia de morte ab leucemia in radiologos in le Statos Unite pro le decennio ab 1949 ad 1958 esseva circa quatro o cinque vices plus grande que in medicos non-radiologic.

2. Isto es approximativemente un medietate del differentia que existeva in le duo precedente decennios.

3. Il es a expectar que le familiaritate con le problema e le uso de adequate protectiones per le radiologos va resultar in un reduction additional de ille differentia, sin unquam abolir lo completemente.

4. Il existe nulle dubita con respecto al relation causal inter radiation ionisante e certe casos de leucemia. Es presentate un revista del major typos de evidentia in supporto de iste relation. Le evidentia le plus positive veni ab le laboratorio experimental, ab collectiones de datos relative al bombardamentos atomic, ab studios in radiologos, e ab le roentgeno-therapia in casos de spondylitis ankylosante. Evidentia de fortia multo suggestive veni ab le irradiation del thymo infantil. Evidentia de valor dubitose es illo associate con le uso therapeutic de iodo radioactive e con irradiation diagnostic fetal ante nato.

Le majoritate del expertos opina que le evidentia supporta le existentia de un relation linear inter dose e responsa, sed le concepto de un relation non-linear es etiam supportate con bon autoritate. Si il existe un relation linear pro micre doses o si il existe un dose limine remane un question controverse.

BERYLLIUM DISEASE: A CONTINUING DIAGNOSTIC PROBLEM*

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The potential danger of radium deposited in the body came to public and professional attention in a dramatic fashion in the 1920's (Blum[2]), when girls tipping their brushes in a luminizing paint solution were discovered to be suffering from osteitis, profound anemia, and in some cases osteogenic sarcoma (Castle, Drinker and Drinker[3]). In a remarkably parallel fashion, in the early 1940's a number of young women engaged in fluorescent lamp manufacture developed a clinical syndrome of recognizable consistency which proved to be related to the inhalation of certain beryllium compounds (Hardy and Tabershaw[14]). Many of these cases also were dramatic. The wish to guard trade secrets and avoid compensation claims led to confusion in delineating this industrial illness. European literature of the 1930's (Gelman[11], Meyer[18], Weber and Englehardt[23]) had described disease in several beryllium-using industries. A number of animal studies done for a variety of reasons in laboratories here and abroad proved many beryllium compounds to be toxic. These data were not utilized or were misinterpreted in the United States prior to 1945 (Hyslop *et al.*[16]). In this year, Van Ordstrand[21] described an acute reaction to acid salts of beryllium, which reversed when exposure ceased. Such exposures, if intense, produced a chemical pneumonia with such a Roentgen-ray picture, and resulted in fatal pulmonary edema in a few cases.

Less intense exposures of varying duration to certain toxic compounds may cause both a subacute (Aub and Grier[1]) and chronic form of beryllium poisoning in workers (Hardy and Stoeckle[13], Hardy and Tepper[15]) and in neighbors (Chesner[4]). It is the diagnostic criteria of the chronic disease which are here discussed. The reasons for presentation of this knowledge are several. The diagnosis of chronic beryllium intoxication may be difficult. The clinical character is very like other diseases, notably sarcoidosis, and also miliary tuberculosis, plus the vast array of diseases presenting miliary densities on chest roentgenogram with and without such nonspecific symptoms as dyspnea and cough. In addition, there may be a delay in onset between illness (Table 1) and the last exposure

TABLE 1.—DELAY IN ONSET—CHRONIC DISEASE

	No. of Cases
Less than 1 month	126
1 month to 1 year	27
1 to 5 years	89
5 to 10 years	56
More than 10 years	12

(Beryllium Registry Data)

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to toxic beryllium compounds varying in time to as long as 5 to 10 years and in a few cases longer. A Registry of cases of beryllium disease begun in 1951 now has on file 650 records, and here the help of the many participating physicians is gratefully acknowledged. Forty cases have been entered since January, 1959; 11 of these during 1959, 27 during 1960, and 4 since January, 1961. This continuing appearance of cases is associated with the striking delay in onset as well as the spreading awareness of the disease.

TABLE 2.—BERYLLIUM USES

Fluorescent lamps	Aircraft manufacture
Neon signs	Electronics (Roentgen-ray tubes)
Nuclear reactors	Alloys (with Cu, Al, steel)
Space vehicles	Bicycle wheels
Guidance systems components	Fishing rods

TABLE 3.—BERYLLIUM-USING OPERATIONS

Beryllium Metallurgy	Be compounds—manufact.
Be Extract.—all operations	Atomic energy development
Be Ceramic—mixing, pressing	Alloy manufact.—melting, casting
Fluor. powder—mixing	Alloy use—machining, grinding
Fluor. lamp manufacture—Coating, sealing, salvage	Neon signs—coating, cutting, repair

Another important reason for discussing beryllium poisoning is the ignorance of physicians and patients of the *quality* and *quantity* of work exposure which may be responsible for the illness. One needs to know that beryllium has been useful (Table 2) in many ways in industry and will be increasingly so as man explores space (Kaufman[17]). This is because when alloyed with other metals it imparts lightness, hardness, and resistance to heat and stress. It is a source of neutrons when bombarded with alpha particles. To give a notion of the demand for beryllium, requirements increased production of the ore from less than 1,000 tons in 1938 to 12,500 in 1956, and plans call for an increase of this total to 20,000 tons a year. The list of uses for beryllium gives an idea of its widespread value to industry and research. The variety of operations (Table 3) which may be involved in beryllium usage illustrates the need to question a worker-patient in detail.

How does a case of chronic beryllium disease present itself? The initial picture is usually one of pulmonary involvement. This may be a discovery by chance of densities seen in a routine chest roentgenogram, or patient complaint of dyspnea, cough, and weight loss leading to chest roentgenogram. In a number of cases manifest clinical illness apparently follows such a stress as pregnancy, infection, or surgery. This listing of symptoms (Table 4) from the Registry cases shows these to be not unique for a single disease. The objective findings and the recorded complications (Table 5) present evidence that toxic beryllium effect produces a systemic intoxication with involvement of several organs and systems, not exclusively localized pulmonary lesions (Hall *et al.*[12]).

Further evidence of the systemic character of this chemical intoxication (Table 6) is provided in this compila-

tion of laboratory indices that may be abnormal as, for example, changes in serum proteins and hypercalcuria leading to calculi.

Chronic beryllium disease presents a wide spectrum of clinical pictures. A small number of patients, followed for several years, continue to have such biroentgenogram, enlargement of liver and spleen, and stone formation, who go through uncontrollable bouts of fever, and remain dependent on steroids and oxygen therapy for existence. This chronic poisoning is a disease of long course with remissions and exacerbations. For some reason, the

TABLE 4.—SYMPTOMS (PERCENTAGE AS OBSERVED IN 650 REGISTRY CASES, 1959)

Dyspnea		Cough	
On exertion	69	Nonproductive	45
At rest	17	Productive	33
		Chest pain	31
Weight loss		Anorexia	26
More than 10%	46	Weakness	17
0 to 10%	15	Joint pains	8.5

TABLE 5.—SIGNS (PERCENTAGE OBSERVED IN 650 REGISTRY CASES, 1959)

Chest signs	43		
Cyanosis	42	Complications	
Clubbing	31	Cardiac failure	17
Hepatomegaly	5	Renal stone	10
Splenomegaly	3	Pneumothorax	12

(Signs are not included when they are attributable to cardiac failure)

TABLE 6.—SUPPORTING LABORATORY EVIDENCE

Chest Roentgen-ray densities involving all lung fields
Alveolar capillary block on lung function study
Increase in total proteins
Relative increase in gamma globulin
Hypercalciuria
Evidence of right heart strain
No change in tuberculin reactivity
Increase in hematocrit
Disturbed liver function
Negative nitrogen balance

lateral chest roentgenographic changes *only*. There are other patients with mild to moderate disability, complaining of cough, dyspnea, weakness, who have varying degrees of diffusion defect with resulting oxygen unsaturation. There are still other patients, completely disabled, with obvious serious lung destruction as reflected in the

55 cases of illness occurring among neighborhood women and children, with a 60% mortality, demonstrate this to be a more serious form of the disease than the worker illness, with 28% mortality (Peyton and Worcester[19]). It is interesting that of the neighborhood patients, some became ill by inhaling beryllium because of living

within ½ mile of a plant which took no precautions to control the hazard; some were exposed to heavily contaminated clothes; and some to a combination of these two sources of toxic materials.

Favorable response to continuous use of steroids is the rule, but to date follow-up of treated cases with lung function study and close clinical observation shows slow but relentless progression of the disease in most cases and little evidence of complete recovery. As might be expected, the most frequent cause of death is cor pulmonale. Pulmonary insufficiency is next, and complicating infection is reported as third most important. There are few cases of tuberculosis in the documented chronic beryllium series, even though many patients spent months exposed to active acid-fast infection in sanatoria due to mistake in diagnosis. There are only 3 cases of cancer of the lung in the 650 Registry cases despite the evidence that beryllium produces pulmonary cancer in laboratory animals.

The difficulty in establishing with certainty a diagnosis of beryllium poisoning can be emphasized by 3 cases:

Case Reports. CASE 1. A 27-year-old man spent some months in a local tuberculosis sanatorium because of chest roentgenographic changes and cough, during which time no evidence of tuberculosis was established. Because he lived near a beryllium-using plant, he was referred for study with a diagnosis of neighborhood beryllium poisoning. Lung biopsy showed granulomatous disease but no beryllium present. Within a year the chest roentgenogram became normal, confirming the final diagnosis of sarcoidosis.

CASE 2. The case of C.M., a 34-year-old woman, reported in full by Sprague and Hardy[20] is representative of a small series suggesting that cases of beryllium disease may go unrecognized. Because of joint pains, weakness, cough, dyspnea, low grade fever, weight loss, and a cardiac murmur a diagnosis of rheumatic heart disease was made. Miliary densities in both lungs, apex to base, seen on roentgenogram coupled with a history of eighteen months' exposure to beryllium compounds in fluorescent lamp manufacture suggested a diagnosis of beryllium poisoning. Skin lesions, indistinguishable from those of Boeck's disease, were present and when biopsied revealed non-caseating granulomata and beryllium in the tissue by spectrographic analysis, confirming the diagnosis. Eight years later, while improved, the chest roentgenogram is not clear and dyspnea on effort requires steroid therapy.

CASE 3. A 42-year-old draftsman had worked 6 months in a fluorescent lamp manufacturing plant where he was heavily exposed to beryllium compounds during the time it was held that no hazard existed. He suffered cough, dyspnea, weight loss, joint pains, weakness for 9 months. Because of these symptoms and an abnormal chest roentgenogram a variety of diagnoses: asthma, polycythemia vera, bronchiolitis obliterans, sarcoidosis, were advanced. He showed cyanosis and clubbing and lung function study demonstrated the diffusion defect of a marked alveolar-capillary block. Biopsy, with beryllium detected in the tissue, made certain the diagnosis of chronic beryllium disease. After 9 years on steroid drugs this man, while still dyspneic on effort, and without change in his roentgenogram, is greatly improved and works steadily.

These cases help to illustrate the fact that the diagnosis rests primarily on establishing such exposure to toxic beryllium compounds as a considerable body of evidence shows to be enough to produce disease. Strong epidemiologic evidence or finding beryllium in tissue or urine, or both, are the means at hand (Cholak[5], Cholak and Hubbard[6]). Such evidence of toxic exposure with the described supporting clinical and laboratory data confirm the diagnosis.

Curtis has suggested[7,8] a beryllium patch test to aid diagnosis. There is not agreement that a negative test rules out beryllium poisoning. In addition, there is some evidence that use of such a test may exacerbate (Waksman[22]) toxic beryllium effect.

It is important to emphasize that knowledge of this occupational disease,

its natural history, clinical variants, prognosis, and complications, is still accumulating, so that it is not possible to speak now with finality of all diagnostic criteria.

Pathologists do not agree that there are unique characteristics of beryllium lesions. Freiman points out that the similarity between the pathological picture of farmers' lung, due to inhalation of mouldy hay, and that of chronic beryllium poisoning emphasize the fact that material from biopsy and necropsy is only one criterion for diagnosis (Frank[9], Freiman[10]).

TABLE 7.—DIFFERENTIAL POINTS, BOECK'S AND BE DISEASE IN BE DISEASE TO DATE: (1961)

No hilar adenopathy alone
No ocular involvement
No uveoparotid fever
No cystic bone changes
Low rate of active tuberculosis
No change in tuberculin reactivity
Less favorable prognosis
Slower response to steroids

Thus, differential diagnosis between beryllium disease and sarcoidosis, the most difficult diagnostic problem, at present rests on the establishment of beryllium exposure. However, there remain several striking differences between the two diseases, although further experience with the occupational illness may alter these (Table 7). There is no change in reaction to tuberculin as in sarcoidosis. No eye, tonsil, parotid, or cystic bone lesions have as yet been reported in the chemical intoxication and no case of beryllium poisoning with hilar adenopathy alone has been reported. Increase in knowledge of both sarcoidosis and beryllium disease may alter the present fact that the former appears to carry a better prognosis and more favorable, lasting response to steroids.

This communication is intended to draw attention to the present state of knowledge of a man-made disease of relatively recent description in the United States. Current and intended use of potentially harmful compounds, delay in onset of beryllium-related illness, and similarity to other granulomatous disease (especially sarcoidosis) make beryllium poisoning a continuing diagnostic problem.

Summary. Reports of disease believed related to exposure to toxic beryllium compounds appeared in European medical literature in the early 1930's. In the United States, the development of fluorescent lamp manufacture requiring the use of certain beryllium compounds resulted in job-related illness recognized in the middle 1940's. The decision to use the element and its alloys in nuclear reactor development, space vehicles, and guidance systems, has created a demand for knowledge of the harmful effects of toxic beryllium compounds and their prevention.

A Registry of recognized cases of beryllium disease begun in 1951 now has on file 650 records received from physicians. About 24% of these patients are dead. Forty-two cases have been entered in the Registry since January, 1959: 11 in 1959, 27 in 1960, and 4 since January, 1961. These 650 cases arise from more than 70 companies, representing a dozen different operations.

The diagnosis of the chronic form of beryllium disease may be difficult. Signs and symptoms are not unique and there may be a delay in onset of manifest illness from the last beryllium exposure of as long as 15 years. In addition, chronic beryllium poisoning mimics other diseases, most strikingly sarcoidosis (Table 7), a clinical entity recognized more frequently in recent years in this country.

Since beryllium disease has been documented in the United States for only 15 years, its natural history, clinical variants, prognosis, and therapy are as yet not well understood. In addition, likely sources of toxic exposures are described (Table 3) to aid the clinician in diagnostic evaluation of occupational histories of patients suspected of having chronic beryllium poisoning.

REFERENCES

1. **Aub, J. C.,** and **Grier, R. S.:** J. Indust. Hyg. & Toxicol., **31,** 123, 1949.
2. **Blum, T.:** Osteomyelitis of the Mandible and Maxilla, Am. Dental Assn., September, 1934.
3. **Castle, W. B., Drinker, K. R.,** and **Drinker, C. K.:** J. Indust. Hyg. & Toxicol., **7,** 371, 1925.
4. **Chesner, C.:** Ann. Int. Med., **32,** 1028, 1950 (Abstracted J. Am. Med. Assn., **144,** 577, 1950.
5. **Cholak, J.:** Arch. Indust. Health, **19,** 205, 1959.
6. **Cholak, J.,** and **Hubbard, D. M.:** Analyt. Chem., **20,** 73, 1948.
7. **Curtis, G. H.:** Arch. Dermat. & Syphil., **64,** 470, 1951.
8. Idem: Arch. Indust. Health, **19,** 150, 1959.
9. **Frank, R. C.:** Am. J. Roentgenol., **79,** 18, 1958.
10. **Freiman, D. G.:** Arch. Indust. Health, **19,** 188, 1959.
11. **Gelman, I.:** J. Indust. Hyg. & Toxicol., **18,** 371, 1936.
12. **Hall, T. C., Wood, C. H., Stoeckle, J. D.,** and **Tepper, L. B.:** Arch. Indust. Health, **19,** 100, 1959.
13. **Hardy, H. L.,** and **Stoeckle, J. D.:** J. Chron. Dis., **9,** 152, 1959.
14. **Hardy, H. L.,** and **Tabershaw, I. R.:** J. Indust. Hyg. & Toxicol., **28,** 197, 1946.
15. **Hardy, H. L.,** and **Tepper, L. B.:** J. Occupational Med., **1,** 219, 1959.
16. **Hyslop, F., Palmes, E. D., Alford, W. C., Monaco, A. R.,** and **Fairhall, L. T.:** Nat. Inst. of Health Bull. No. 181, U.S. Public Health Service, 1943.
17. **Kaufman, A. R.:** Arch. Indust. Health, **19,** 91, 1959.
18. **Meyer, H. E.:** Beitr. z. Klin. d. Tuberk., **98,** 388, 1942.
19. **Peyton, M. F.,** and **Worcester, J.:** Arch. Indust. Health, **19,** 100, 1959.
20. **Sprague, H. B.,** and **Hardy, H. L.:** Circulation, **10,** 129. 1954.
21. **Van Ordstrand, H. S., Hughes, R., DeNardi, J. M.,** and **Carmody, M. G.:** J. Am. Med. Assn., **129,** 1084, 1945.
22. **Waksman, B. H.:** Arch. Indust. Health, **19,** 154, 1959.
23. **Weber, H. H.,** and **Englehardt, W. E.:** Zentralbl. f. Gewerbehyg., **10,** 41, 1933.

SUMMARIO IN INTERLINGUA

Morbo a Beryllium: Un Continue Problema Diagnostic

Reportos de maladia presumptivemente relationate al exposition a toxic compositos de beryllium appareva in le litteratura medical de Europa durante le prime annos post 1930. In le Statos Unite, le disveloppamento del fabrication de lampas fluorescente (que require le uso de certe compositos de beryllium) resultava in maladia a base occupational, primo recognoscite al medio del quinte decennio. Le decision de usar le elemento e su alligatos in le disveloppamento de reactores nucleari, de vehiculos cosmonautic, e systemas de commanda ha create un demanda pro informationes in re le effectos nocive del toxic compositos de beryllium e le possibilitate de su prevention.

Un Archivo de recognoscite casos de morbo a beryllium, mantenite depost 1951, contine hodie 650 protocollos recipite ab medicos. Circa 24% de iste patientes es morte. Quaranta-duo casos representate in le Archivo esseva reportate depost januario 1959, i.e. 11 in 1959, 27 in 1960, e 4 depost januario 1961.

Iste 650 casos proveni ab plus que 70 companias que representa un dozena de differente processos.

Le diagnose del forma chronic de morbo a beryllium es a vices difficile. Le signos e symptomas non es distinctive, e il pote occurrer un retardo in le declaration manifeste del morbo per usque a 15 annos a partir del ultime exposition a beryllium. In plus, chronic invenenamento per beryllium simula altere morbos, incluse—como exemplo particularmente frappante—sarcoidosis que es un entitate clinic recognoscite plus frequentemente in recente annos in iste pais.

Viste que morbo a beryllium es documentate in le Statos Unite solmente depost 15 annos, su historia natural, su variantes clinic, su prognose, e su therapia es non ancora ben comprendite. In plus, fontes probabile de exposition toxic es describite, como adjuta pro le clinico in su evalutation diagnostic del antecedentes occupational de patientes in qui il existe le suspicion de chronic invenenamento per beryllium.

SUMMARIO IN INTERLINGUA

(See page 211 for original article)

Disgranulation del Basophilo in le Homine, Inducite per Lipemia Alimentari

Nos ha trovate que lipemia postprandial es associate specificamente con disgranulation del basophilos. Tal disgranulation basophilic es reguardate como le mechanismo del liberation physiologic de heparina e ergo como importante in le reaction del clearing. In plus, lecithinase A es reguardate como possibilemente interessate in iste mechanismo.

Viste que le leucocyto basophilic es un cellula delicate e rar, un methodo special ha essite empleate in su manipulation. Le methodo consiste del fixation de 0,1 ml de sanguine venose in un fixativo acido-alcoholic. Isto destrue le erythrocytos, de maniera que le leucocytos remane suspendite in un solution clar. Le leucocytos es alora concentrate per medio de un filtro de cellulosa, e le basophilos es tincturate specificamente. Le filtro con le leucocytos es montate e quaranta consecutive basophilos es categorisate in distincte gruppos a base de lor dimensiones, lor proprietates tincturatori, e le location de lor granulos basophilic.

Un respasto de butyro (4 g per kg) causava disgranulation del leucocytos basophilic intra 2 a 3 horas in circa 75% del subjectos studiate. Nivellos de triglycerido de 8 mEq per L o plus esseva associate regularmente con grados significative de disgranulation basophilic. Esseva trovate nulle correlation inter le grado de disgranulation e le nivellos de phospholipido o cholesterol. Margarina, que es ric in non-saturate acidos grasse, monstrava le mesme effecto disgranulatori como butyro. Repastos consistente primarimente de hydratos de carbon o de proteinas esseva sin effecto significative. Le mesmo valeva pro un dose unic de cortisona (200 mg).

BETA-HEMOLYTIC STREPTOCOCCAL PNEUMONIA: REPORT OF AN OUTBREAK IN A MILITARY POPULATION*

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The attention of the present day physician is directed to the beta-hemolytic streptococcus mainly in its relation to pharyngitis and rheumatic fever, with an occasional reference to glomerulonephritis. Except for World Wars I and II, little mention is made of pneumonia caused by this organism. Standard textbooks of medicine and pathology speak of it only in briefest form. That it may yet be of more than historical interest was brought forcibly to our attention during the winter months of 1959-1960, when 20, young, previously healthy Navy men developed pneumonia caused by this agent. One, the first, died of it.

There is a rather predictable incidence of bronchopneumonia in the young recruit population at this installation which numbers about 10,000. During the peak season, approximately 200 hospital admissions monthly are seen with pneumonia. In nearly all of these, white blood counts are under 10,000 per c.mm. and throat and sputum cultures fail to grow pathogenic bacteria. Despite unsuccessful efforts to isolate any significant number of viruses, about 95% are presumed to be viral in etiology.

However, the following fulminating case of beta-hemolytic streptococcal pneumonia alerted us to the problem.

Case Reports. CASE 1. F.J.G.: This 17-year-old, Seaman Recruit, had been well until the evening prior to the day of admission, October 12, 1959, when he developed fever, cough, left chest pain and a generalized erythematous rash. He was hospitalized at his dispensary where the rash was felt to be that of rubella, and then transferred to the contagion ward of this hospital. Physical examination on admission showed a well-developed, well-nourished, comfortable, oriented male, with an oral temperature of 101.8° F., a moderately injected pharynx, and rales over the left lower lung field. Laboratory work on admission revealed the white blood count to be 19,800 per c.mm. with a "shift to the left" in the differential count. The erythrocyte sedimentation rate was 46 mm. per hour. The urinalysis showed 10 mg. per 100 ml. protein. The admission chest film demonstrated a left lower lung field pneumonia with minimal pleural effusion.

During the first 2 days of his hospital course, he did fairly well, but he continued to be febrile and to complain of left chest pain on deep breathing and coughing. On the morning of the third hospital day, he developed respiratory distress, his extremities became cyanotic, and his blood pressure fell. At this time, culture of the throat obtained on admission was reported to show beta-hemolytic streptococcus, group A. Penicillin and erythromycin, along with levophed were

*The opinions expressed herein are those of the authors and do not necessarily reflect the views of the Navy Department.

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Fig. 1

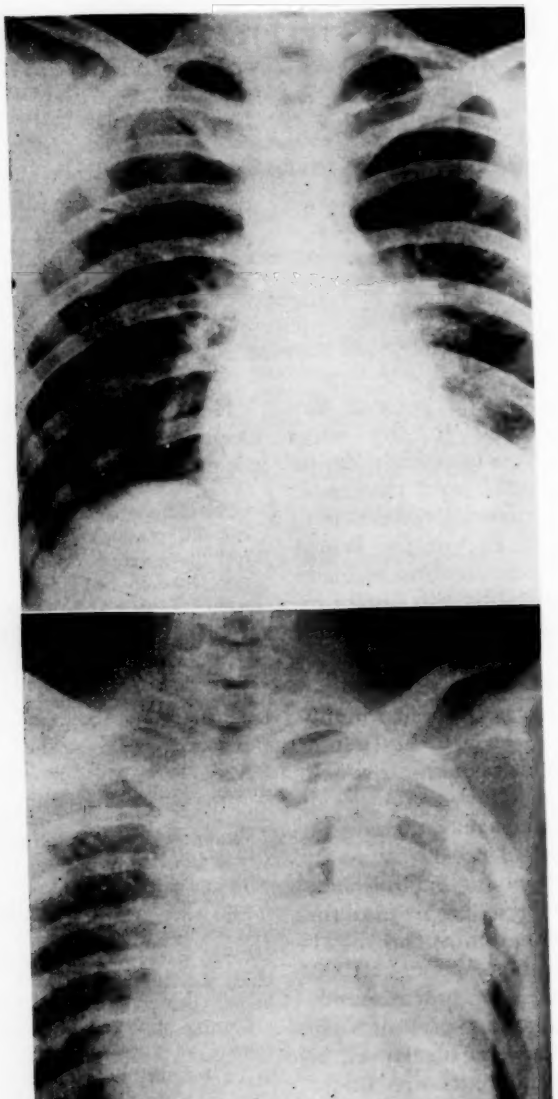

Fig. 2

Fig. 1.—Portable roentgenogram of the chest in Case 1 on October 13, 1959, showing extensive pneumonic infiltration in association with minimal pleural effusion in the left lower lung field.

Fig. 2.—Portable roentgenogram of the chest in Case 1 on October 15, 1959, after left thoracentesis shows a pneumothorax with partial collapse of the left lung. The line of pneumothorax is seen extending along the left lateral wall into the left apex and along the mediastinum medially.

given intravenously. A repeat chest film showed the left chest to be almost completely obliterated. Thoracentesis yielded 1000 ml. of greenish-yellow fluid. He continued a downhill course, became comatose, and expired during the late afternoon of that day. Preceding death, the above noted rash had become confluent, erythematous, and blanched on pressure—more like that of scarlet fever. Culture of the pleural fluid grew beta-hemolytic streptococci (see Figs. 1 and 2).

Necropsy Findings: Gross: External examination was unremarkable except for generalized mottled cyanosis and a small thoracotomy incision in the left lateral mid-chest area at the site of surgical drainage. In the left pleural cavity, there was no free pus or fluid; however, all the pleural surfaces were coated by a thick layer of fibrinopurulent exudate. The pericardial and right pleural cavities were uninvolved. The spleen, kidneys, and liver were increased in weight by about a third, and the cut surfaces of the liver and kidneys were pale. The collecting systems of the kidneys appeared normal. Lymph nodes were enlarged and soft generally. The heart was entirely free of gross lesions.

The main gross postmortem findings were found in the lungs. The right lung weighed 850 gm. and the left 1560 gm. The left lung was completely consolidated and airless, while the right lung was edematous, but not grossly pneumonic. The bronchi, especially on the left, contained much bloody purulent fluid. The cut surface of the left lung was striking in appearance, with the bronchi and their finest branches prominently outlined due to their engorgement by pus, and separated by lung parenchyma varying in color from dark red to tan. The left lower lobe was particularly impressive, the proximal one-half being dark red and moderately firm and sharply demarcated from the light tan, completely consolidated peripheral zone. The right lung on section showed only edema and congestion, and unlike the left lung, its bronchi were not clogged with pus. Postmortem cultures taken from the left lung, pleural cavities, and heart blood showed no growth. However, the patient had received massive doses of penicillin during the last day of life.

Microscopic: Minimal changes attributable to the probable septicemia were found in the heart and liver. The left ventricle contained small focal infiltrations of neutrophils, plasma cells, and a few eosinophils and histiocytes. These were just beneath the endocardium and around small blood vessels in the myocardium. No Aschoff-type cells were noted. Similar infiltrates were found in the portal areas of the liver.

The kidneys were involved by an acute interstitial nephritis as described by Mallory[9] in streptococcal septicemias. This was characterized by the presence of neutrophils and mononuclear cells in the interstitium, glomerular spaces and capillaries, and tubular lumens. The tubular epithelium had pale, swollen cytoplasm.

Only edema and congestion were found in the right lung, although many of the bronchi and bronchioles contained pus. In the left lung, the bronchi and bronchioles were filled with exudate, and in some cases a necrotic membrane replaced the mucosa. Some bronchial walls were thickened by edema, engorged blood vessels, and mononuclear cell infiltration, while many bronchioles had dilated lumens and thin walls, making differentiation from dilated pus-filled lymphatics difficult. The alveoli generally contained edema fluid, fibrin, and cellular exudate, and in a good many areas proliferating alveolar lining cells were noted, sometimes associated with interalveolar organization of the exudate. Septa were thickened and showed vascular congestion and infiltration by neutrophils and mononuclears. One of the striking features was the purulent engorgement and dilatation of lymphatic channels, particularly near the pleural surfaces. The pleural surface itself was covered by a thick layer of organizing fibrin. The interlobular septa were considerably widened due to serofibrinous edema. Sections taken from the grossly dark-red areas in the left lower lobe showed intense vascular congestion and alveolar hemorrhage with relatively scant exudate. No evidence of any type of viral infection, such as measles, was found in any of the sections examined (see Figs. 3, 4, 5 and 6 below).

DISCUSSION OF PATHOLOGICAL CHANGES. Cole and MacCallum[3], in 1919, in their study of a large number of pneumonia cases in Army Camps during World War I gave us the only detailed account of the pathologic features of streptococcal pneumonia. In the ensuing years virtually nothing has been added to their description. They pointed out that the dominant features were those of an interstitial bronchiolar pneumonia as manifested by thickening of the bronchiolar walls

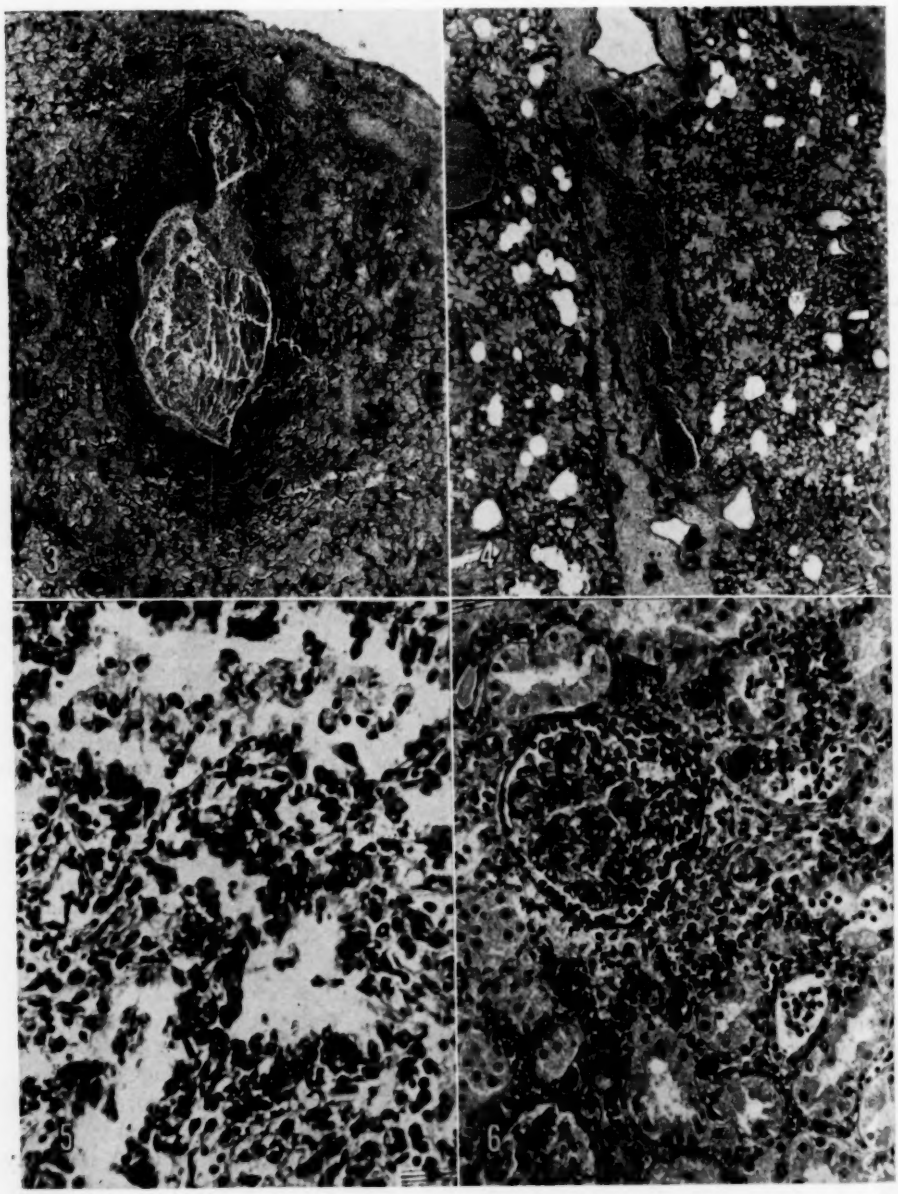

Fig. 3.—Lung (×15). In the center is a dilated bronchiole filled with pus and lined by a necrotic membrane. At the top is the fibrin-covered pleura. The alveoli contain edema fluid, blood, and exudate.

Fig. 4.—Lung (×15). Running vertically in the photomicrograph is a widened, edematous interlobular septum that contains very prominent pus-filled lymphatic channels.

Fig. 5.—Lunch (×300). The alveoli here have a prominent "metaplastic" lining epithelium. Clumps of macrophages can be seen in the alveolar space at the top.

Fig. 6.—Kidney (×200). Glomerulus containing many leukocytes in the glomerular space and the tuft itself. Below and to the right of the glomerulus is a tubule which also contains leukocytes.

by mononuclear cells and hyperemic vessels and, frequently, diffuse necrosis of the mucosal lining. Also emphasized were other findings well demonstrated in our case: purulent engorgement of lymphatics, rapidly organizing fibrinopurulent pleural exudate, and partially organizing parenchymal exudate with thickened septa and large numbers of proliferating alveolar lining cells. It seems likely that the striking involvement of lymphatics is responsible for the early pleurisy, presumably by means of retrograde spread of the infection.

A second type, called lobular pneumonia, was mentioned by MacCallum. It is characterized by areas of consolidation with extensive necrosis and large numbers of bacteria, with no interstitial reaction. This type would seem to be merely an example of overwhelming infection with little host resistance.

Many of the cases described in this monograph had clinical evidence of a preceding measles infection, in fact, in some instances the streptococcal pneumonia outbreaks followed closely behind in the shadow of the measles epidemics. However, MacCallum makes no mention of any histological features in these necropsies that could be attributed to measles.

MacCallum does not dwell at any length on changes in other organs secondary to streptococcal bacteremia or septicemia. However, Mallory lists focal cellular infiltrates in the heart, liver, and kidneys as characteristic features of this disease. More important is the renal lesion which he terms "acute interstitial nephritis." The kidney findings in our case are very similar to those mentioned by Mallory and resemble those of an early acute hematogenous pyelonephritis. Neither this change nor the focal organ infiltrates can be considered specific for streptococcal infection, however.

The second case is quite typical of the cases in this series.

CASE 2. R.B.D.: This 20-year-old, Seaman Recruit, had had symptoms of a mild upper respiratory infection intermittently for the past few weeks prior to admission, January 22, 1960. On the evening of the day prior to admission, he developed cough, fever, and left chest and shoulder pain on deep inspiration. He denied sore throat. A chest film taken at his dispensary on the day of admission showed a diffuse left lower lobe infiltrate. He was referred to this hospital. Physical examination showed a young man complaining of left shoulder pain on breathing deeply. His temperature was 102° F. orally. The pharynx was moderately injected; the tonsils were covered by a white exudate. Bilateral basilar rales were heard over the chest. Cervical adenopathy was present. Laboratory studies on admission showed a white blood count of 12,400 with a shift to the left on differential count and the erythrocyte sedimentation rate was 25. The chest film on admission showed a moderate left lower lobe infiltrate with associated pleural effusion considered to be in excess of 500 ml. The fever continued for 9 days. The day following admission he was placed on chloramphenicol and erythromycin. Throat culture grew beta-hemolytic streptococci. Eight hundred milliliters of creamy yellow fluid were removed by thoracentesis 2 days after admission. This grew beta-hemolytic streptococci, group A, type 14. A closed thoracotomy tube was kept in place for 3 days with resultant improvement both in his clinical condition and chest roentgenogram. He had a 20 pound weight loss during his illness, however, and residual left pleural thickening was seen on roentgenogram. He was discharged to duty 2 months after admission.

COMMENT. This man's clinical course was dominated by pleuritic pain, fever and dyspnea. The process involved the left chest only. The responsible organism was beta-hemolytic streptococcus group A, type 14. He recovered with antibiotics, thoracentesis, and closed thoracotomy with a residual "pleural peel" evident on roentgenographic examination.

The third case is characterized by having a prolonged course.

CASE 3. J.C.S.: This Seaman Recruit, an 18-year-old man, had been well until about

30 hours prior to admission on October 25, 1959, when he developed cough, fever, and moderate left anterior chest pain with respiration. He had been treated for 5 days at his dispensary 3 weeks prior to admission with penicillin and supportive therapy for an upper respiratory infection. On admission, physical examination showed an oral temperature of 105° F. and a pulse of 140. He complained of severe left anterior chest pain with respiration. He was tachypneic and anxious. His throat was fiery red. Decreased breath sounds and rales were heard at the left lung base posteriorly. There was an erythematous rash over the chest and abdomen. Laboratory studies revealed a white blood cell count of 16,800 per c.mm. with a "shift to the left" on differential count. The chest roentgenogram, on admission, showed a left basilar infiltrate with pleural fluid. There were also patches of bronchopneumonia seen at the right base and right upper lobe.

The patient's hospital course showed him to be acutely ill after admission. Penicillin and chloramphenicol were given at the outset with supportive therapy. Thoracentesis was performed on the second and sixth hospital days with the removal of 1810 and 970 ml. of thick yellowish fluid, respectively. A blood culture taken before treatment grew group A beta-hemolytic streptococci. Throat culture on admission also grew this organism and smear of pleural fluid showed Gram-positive diplococci. After 34 days of fever and a 25 pound weight loss, he recovered with residual organized infiltrate at the left base.

Two months after admission, decortication of the left lower lobe was performed with the removal of thick fibrous cortex. He then improved enough to return to duty 4 months after admission (see Figs. 7 and 8 below).

COMMENT. This man had the rapid onset of cough, pleuritic pain, fever and rash. Bilateral but chiefly left pleural effusion developed which responded poorly to treatment. Fever persisted for 34 days. Trapping of the left lung by the residual pleural peel necessitated decortication.

CLINICAL FEATURES. The 20 patients treated here were all Caucasian males between the ages of 17 and 23 years.

Complaints on admission in over half the cases were those of pleuritic pain, cough and sore throat. During the course in the hospital, cough occurred in all patients and was nonproductive in only 3. Pleuritic pain was noted in 18. Only one patient had had a previous bout of pneumonia and 15 had no history of any respiratory difficulty other than common colds.

The most uniform physical findings were fever (over 104° orally in 7), tachypnea, tachycardia, inflamed pharynx and chest findings of pneumonia. (A pleural friction rub was heard in only one case.) The median duration of fever was 8 to 9 days.

Hemograms revealed elevated white blood counts with marked "left shifts" and elevated erythrocyte sedimentation rates. In 11 cases, white blood cells were above 20,000 per c.mm. including 3 over 40,000. No patient showed a hemoglobin less than 10 gm.%, but 9 patients fell below 12.5 gm.% at some time in their illness. Seven patients showed proteinuria. Of these, 4 were reported as "trace."

The disease process had a striking predilection for the left chest. Four right-sided effusions were minimal in extent, whereas all 15 left effusions were of considerable amount. Sixteen patients had residual pleural thickening; 15 of these had readily apparent "pleural peels." Two residual parenchymal infiltrations proved to be areas of bronchiectasis on bronchographic examinations.

Beta-hemolytic streptococci were cultured from the throat in 14 patients, from the sputum in 13, the pleural fluid in 6, and the blood in one. In only 4 cases was the diagnosis made by positive throat culture only. All of these 4 patients had pleural effusion but had received large dosages of antibiotics before thoracentesis. The single patient without pleural effusion had the organism isolated from the sputum. Blood

Fig. 7

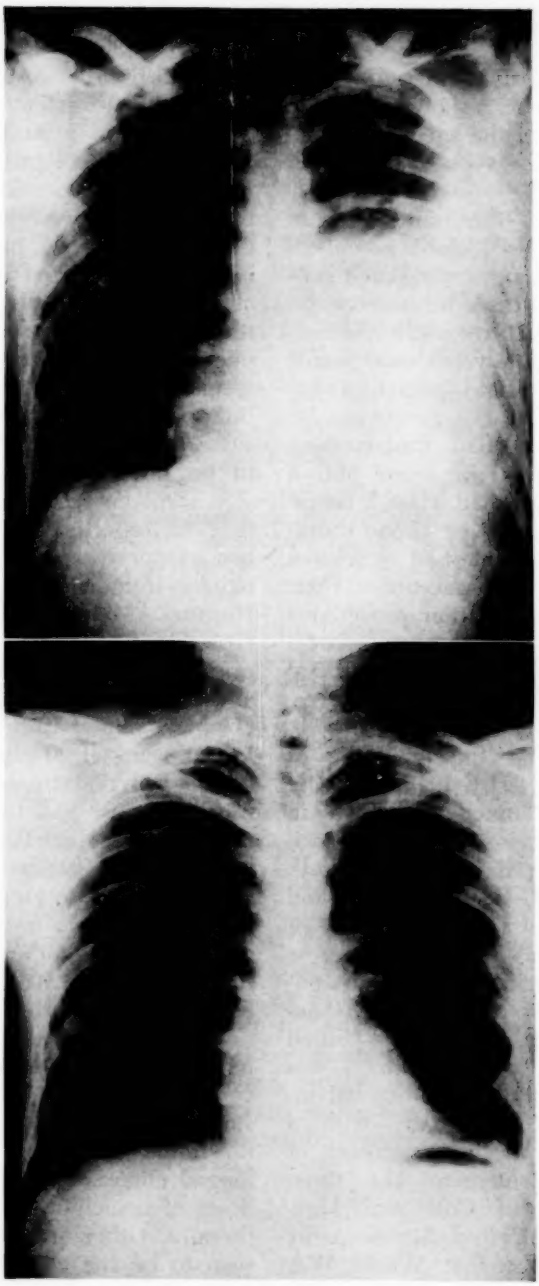

Fig. 8

Fig. 7.—Portable roentgenogram of the chest in Case 3 on October 26, 1959, the day following admission shows fluid in the left chest obliterating all lung detail below the level of the sixth posterior interspace, extending superiorly along the lateral chest wall up to and over the apex, and causing slight displacement to the right of the heart and mediastinal content. Pneumonitis is also seen in the right upper and lower lung fields.

Fig. 8.—Roentgenogram of the chest in Case 3 on February 8, 1960, eight weeks after decortication procedure on the left. There is minimal residual pleural thickening in the left chest.

cultures were obtained in only 2 patients. One of these 2 showed positive cultures. Typing of the agent was done in 2 cases. Group A, type 14 was found in both cases.*

Penicillin was the antibiotic of choice, but was used alone in only 8 cases. Other antibiotics were used concurrently in most cases because of the severe clinical picture while cultures were pending. In only one case was it necessary to withhold penicillin because of allergy.

Fifteen patients had thoracentesis performed, 7 had 3 or more, and 4 additional patients had closed thoracotomies after the first or second thoracentesis. One patient had a closed thoracotomy before thoracentesis. Four patients had decortication procedures of pleural peel after the acute process had subsided. Discussion of surgical management of these cases, however, is beyond the scope of this paper.

Discussion. That the incidence of pneumonia caused by beta-hemolytic streptococcus is much less than in former years seems to be an established fact (Austrian[1], Finland[5], Kirby[8]). In 1927, Cecil, Baldwin and Larsen[2] attributed approximately 4% of 2000 cases to this organism, whereas in more recent years, it has been considered to be less than 1% of all bacterial pneumonias. In the United States, during both World Wars, it was well recognized that streptococcal infections increased markedly with a concomitant rise in the occurrence of streptococcal pneumonia. The time-honored studies of Cole and MacCallum in the United States Army Camps during the first World War cited above focused attention on this organism as a serious and often fatal secondary invader with measles and influenza. Subsequent studies (Austrian[1], Cecil, Baldwin and Larsen[2], Hall[6], Kirby[8]) have supported the concept that the pneumonia caused by this organism is most often observed following another primary illness.

Reports of primary streptococcal pneumonia are well documented in the literature. However, most of these cases have been observed during epidemics of streptococcal infections in which the majority of these patients with pneumonia were considered to have a secondary pneumonitis. In 1941, Keefer, Rantz and Rammelkamp[7] reported 55 cases of which only 10 were thought to be primary, without evidence of significant antecedant illness. Durkee[4], in 1945, reported a rather large number of primary streptococcal pneumonias from a Public Health Service Hospital. McFarland[10], in 1946, discussed his wartime experience with primary disease observing from 30 to 40 cases out of a total of 4000 cases of pneumonia of streptococcal etiology. In our series of 20 cases caused by the beta-hemolytic streptococcus, all but 2 at the most had little or no recognizable antecedent illness. One patient was admitted with a normal chest roentgenogram to another ward at this hospital and became ill 2 weeks after admission. The other (Case 1) was thought to have had rubella initially. Six other cases were seen with the same clinical course at this time, having pleural effusion but no organisms could be cultured from their throat, sputum, or pleural fluids. Our cases illustrated once more the rather prolonged clinical course even with large doses of penicillin and other antibiotic therapy. Other striking features were seen to be the almost uniform occurrence of pleuritic pain. The predominantly left-sided involvement was noted; significance of this is not determined. Nineteen of 20 cases had significant pleural effusion, and in the

*Organism typing was performed at Naval Medical Research Unit #4, Great Lakes, Illinois.

2 cases typed, group A, type 14, beta-hemolytic streptococcus was seen. It is once again to be noted that 75% of the patients had significant residual pleural thickening. Two patients had residual bronchiectesis.

Summary. Twenty cases of beta-hemolytic streptococcal pneumonia occurred during an outbreak of streptococcal disease at the Great Lakes Naval Training Center and the U.S. Naval Hospital in the winter of 1959-1960. The disease was abrupt in onset, fulminant in progression, and often prolonged in course. The pathologic changes were characteristic and striking. Contrary to previous reports in the literature, antecedent viral infection was not important in its pathogenesis, since only 2 cases showed evidence of it. Even after two decades of antibiotics the disease is of considerably more than historical interest.

REFERENCES

1. **Austrian, R.:** J. Am. Med. Assn., **163,** 1040, 1957.
2. **Cecil, R. L., Baldwin, H. S.,** and **Larsen, N. P.:** Arch. Int. Med., **40,** 253, 1927.
3. **Cole, R.,** and **MacCallum, W. G.:** Monograph 10, The Rockefeller Institute for Medical Research. Baltimore: The Williams & Wilkins Co., 1919.
4. **Durkee, R. E.:** Connecticut Med. J., **9,** 922, 1945.
5. **Finland, M.:** New England J. Med., **263,** 207, 1960.
6. **Hall, W. H.:** Med. Clin. N. America, **43,** 191, 1959.
7. **Keefer, C. S., Rantz, L. A.,** and **Rammelkamp, C. H.:** Ann. Int. Med., **14,** 1533, 1941.
8. **Kirby, W. M.:** Arch. Int. Med., **96,** 809, 1955.
9. **Mallory, G. K.,** and **Keefer, C. S.:** Arch. Path., **32,** 334, 1941.
10. **McFarland, J. E.:** J. Iowa State Med. Soc., **36,** 481, 1946.

SUMMARIO IN INTERLINGUA

Pneumonia a Streptococcos Beta-Hemolytic: Reporto de un Eruption in un Population Militar

Vinti casos de pneumonia a streptococcos hemolytic beta occurreva durante un eruption de morbo streptococcal al Centro de Trainamento Naval e al Statounitese Hospital Naval de Great Lakes, Illinois, durante le hiberno de 1959 a 1960. Le morbo esseva abrupte in su declaration, fulminante in su progresso, e frequentemente prolongate in su curso. Le alterationes pathologic esseva characteristic e frappante. Per contrasto con previe reportos in le litteratura, le antecedente de infection virusal non esseva importante in le pathogenese: Solmente 2 del casos monstrava signos de un tal. Post duo decennios de antibioticos, le morbo continua posseder un interesse plus que historic.

STUDIES WITH TULAREMIA VACCINES IN VOLUNTEERS*

IV. BRUCELLA AGGLUTININS IN VACCINATED AND NONVACCINATED VOLUNTEERS CHALLENGED WITH PASTEURELLA TULARENSIS

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Previous studies from this laboratory have been concerned with the clinical[9,10] and serologic[11] aspects of experimental tularemia in vaccinated and nonvaccinated volunteers following intracutaneous or respiratory challenge. These studies provided a unique opportunity to investigate the occurrence of brucella agglutinins in tularemia under controlled conditions. Various workers (Eisele[1], Feinberg and Wright[2], Foshay[3], Francis[5], Francis and Evans[6], Poston and Smith[8], Stanfield, Taylor and Morgan[13]) have shown that patients with naturally acquired tularemia can exhibit agglutinins for brucella species, but there is not general agreement as to the frequency with which this serologic cross reaction occurs and its importance from the diagnostic standpoint. The present report is concerned with brucella agglutinin formation in 98 vaccinated and nonvaccinated subjects challenged by the intracutaneous or respiratory route with virulent *P. tularensis*. Ancillary studies in rabbits immunized with killed *P. tularensis* also will be presented.

Materials and Methods. Volunteers received either Foshay killed (phenolized) vaccine or viable attenuated vaccine by methods described previously[9,10]. Procedures used for intracutaneous and respiratory challenge have also been discussed in our earlier reports[9,10]. Brucella tube agglutination tests were carried out by standard methods (Spink *et al.*[12]). Tube antigen was obtained from the Bureau of Animal Industry, Beltsville, Maryland.

Formalin-killed suspension of *P. tularensis*, Strains 38, Schu S-4, 425, 503 and the viable vaccine strain† were used to immunize 3, 3, 3, 2 and 4 rabbits, respectively. All strains were grown on GCBA medium (BBL) for 72 hours, harvested in physiologic saline containing 0.5% formalin, and allowed to remain at 4° C. for 24 hours. After appropriate sterility tests had been completed, the suspensions were washed 3 times with sterile saline and resuspended in a concentration which, when diluted 1:10, was equivalent in opacity to MacFarland tube No. 2. Each rabbit was injected intravenously with 0.5, 1.0, 1.0 and 1.0 ml. on 4 consecutive days, respectively. Blood samples were obtained from the marginal ear vein before immunization and at weekly intervals thereafter. At 7 and 11 weeks after the first immunization, each rabbit received an intravenous booster injection of 0.5 ml. of the same suspension used originally. Curves of antibody production and decline were established in each rabbit by three serologic tests: *P. tularensis* bacterial agglutination and polysaccharide hemagglutination, and *Brucella abortus* bacterial agglutination. Procedures used in per-

*This study was supported under Contract with the U.S. Army Cm1C, Fort Detrick, Frederick, Maryland.

†Kindly supplied by Dr. Henry T. Eigelsbach.

TABLE 1.—*P. TULARENSIS* AND *BR. ABORTUS* AGGLUTININ TITERS IN VOLUNTEERS VACCINATED WITH KILLED VACCINE (FOSHAY).

Subject No.	*Type of Agglutinins**	*Weeks after Vaccination*										
		0	*1*	*2*	*3*	*4*	*5*	*6*	*8*	*10*	*12*	*14*
1	T	10	10	640	640	320	160	160	80	80	80	80
	B	0	0	80	80	40	20	20	10	10	0	0
2	T	0	20	640	640	640	640	ND†	640	ND	320	
	B	0	0	20	20	20	10	ND	10	ND	10	
3	T	0	0	0	320	320	320	ND	ND	ND	40	
	B	0	0	0	20	20	20	ND	ND	ND	10	
4	T	0	0	0	320	320	ND	ND	ND	ND	40	
	B	0	0	0	80	80	ND	ND	ND	ND	0	
5	T	0	0	640	320	320	320	ND	ND	ND	ND	
	B	0	0	40	40	40	40	ND	ND	ND	ND	
6	T	0	0	0	80	80	80	ND	ND	ND	40	
	B	0	0	0	10	20	20	ND	ND	ND	20	
7	T	0	0	0	320	320	320	ND	ND	ND	160	
	B	0	0	0	20	20	20	ND	ND	ND	0	
8	T	0	0	640	640	320	320	ND	320	ND	ND	
	B	0	0	40	40	20	20	ND	20	ND	ND	
9	T	0	0	320	160	80	80	80	ND	ND	ND	
	B	0	0	20	20	20	20	20	ND	ND	ND	
10	T	10	10	320	320	160	160	160	160	160	160	
	B	0	0	0	0	10	10	20	10	10	10	
11	T	0	0	640	160	160	80	80	80	80	ND	
	B	0	0	40	40	40	40	40	40	10	ND	
12	T	0	160	160	80	80	80	80	80	40	40	40
	B	0	10	10	20	20	10	10	0	0	0	0
13	T	0	320	320	320	160	160	160	80	80	40	40
	B	0	40	20	20	20	20	20	20	10	10	10
14	T	0	640	1280	1280	1280	640	640	640	640	ND	
	B	0	0	40	40	40	40	40	40	40	ND	
15	T	0	0	320	320	160	160	160	ND	ND	ND	
	B	0	0	40	40	20	0	0	ND	ND	ND	
16	T	0	0	1280	1280	1280	1280	1280	ND	640	640	640
	B	0	0	40	40	20	10	10	ND	0	0	0

*T = *P. tularensis*; B = *B. abortus*
†ND = Not Done

forming the first two have been described previously (Saslaw and Carhart[11]).

Results. Following vaccination with killed tularemia vaccine, 16 of 43 (37.2%) volunteers exhibited brucella antibodies after 9 to 24 days. Peak titers of 1:20, 1:40 and 1:80 were observed in 8, 6 and 2 volunteers, respectively. In 6 of 16, titers were negative after 1 to 3 months. The appearance of peak titers coincided with peak tularemia agglutination titers which were usually 4 to 32 times greater (Table 1).

After immunization with viable attenuated tularemia vaccine, 5 of 28 (17.9%) men with negative base-lines first showed brucella titers of 1:10 to 1:80 after 24 to 31 days. Their peak titers of 1:40, 1:40, 1:80, 1:80 and 1:320 were observed 24, 31, 30, 52, and 51 days respectively, after vaccination. One other volunteer with a prevaccination brucella titer of 1:20 showed a rise to 1:60 on the 38th day. Thus 6 of 29 (20.7%) showed significant brucella antibody titer rise after immunization with viable tularemia vaccine (Table 2).

Intracutaneous challenge of nonvaccinated volunteers with approximately 10 tularemia bacilli induced brucella antibody peak titers of 1:10 to 1:160 in 3 of 12 (25%) men after

TABLE 2.—*P. TULARENSIS* AND *BR. ABORTUS* AGGLUTININ TITERS IN VOLUNTEERS VACCINATED WITH VIABLE ATTENUATED VACCINE.

Subject No.	*Type of Agglutinins**	*Weeks after Vaccination*											
		0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*10*	*12*	*14*
1	T	0	0	40	40	640	2560	2560	2560	2560	2560	2560	2560
	B	0	0	0	0	0	20	40	40	40	40	40	20
2	T	0	0	80	320	640	640	640	320	320	320	320	160
	B	0	0	0	10	20	20	40	20	10	10	0	0
3	T	0	0	160	640	1280	2560	2560	2560	2560	2560	2560	2560
	B	0	0	0	0	80	160	160	320	320	320	160	160
4	T	0	0	160	640	1280	1280	2560	2560	2560	1280	1280	1280
	B	0	0	0	0	10	40	20	80	40	10	0	0
5	T	0	10	80	640	1280	1280	1280	1280	1280	ND†	ND	ND
	B	0	0	0	20	20	40	40	80	20	ND	ND	ND
6	T	0	0	80	320	640	640	640	640	640	ND	ND	ND
	B	20	20	20	20	80	160	160	80	80	ND	ND	ND

*T = *P. tularensis*; B = *Br. abortus*
†ND = Not Done

TABLE 3.—*P. TULARENSIS* AND *BR. ABORTUS* AGGLUTININ TITERS IN NONVACCINATED VOLUNTEERS AFTER INTRACUTANEOUS AND RESPIRATORY CHALLENGE WITH *P. TULARENSIS*

Subject No.	*Method of Challenge*	*Type of Agglutinins*	*Weeks after Challenge*									
			0	*1*	*2*	*3*	*4*	*5*	*6*	*8*	*10*	*12*
1	Intracutaneous	T	—	—	—	160	320	320	320	320	320	160
		B	—	—	—	80	160	80	160	160	80	80
2	"	T	—	—	—	160	640	640	640	640	640	640
		B	—	—	—	—	80	80	80	80	40	40
3	"	T	—	10	20	160	640	1280	1280	640	1280	640
		B	—	—	—	10	10	10	10	10	10	10
4	Respiratory	T	—	—	20	320	1280	1280	640	640	320	ND†
		B	—	10	20	10	10	10	10	10	10	"
5	"	T	—	10	40	320	320	320	320	160	ND	ND
		B	—	—	—	20	20	20	10	20	"	"
6	"	T	—	—	—	160	640	1280	640	640	640	ND
		B	—	—	—	40	20	10	—	—	—	"

*T = *P. tularensis*; B = *Br. abortus*
†ND = Not Done

3 to 4 weeks. Similarly, 3 of 14 (21.4%), challenged with 10 to 50 tularemia organisms by the respiratory route, exhibited brucella antibodies in titers of 1:10 to 1:40 after 1 to 3 weeks. In all instances, the homologous tularemia titer was higher than the heterologous brucella titer (Table 3).

None of 19 volunteers, who received killed vaccine and were subsequently challenged intracutaneously, showed any alteration in brucella antibodies after challenge. Three who did exhibit brucella antibodies after vaccination showed no titer change and 16 showed no brucella antibodies after either vaccination or subsequent challenge. All 19 exhibited local lesions after challenge (Saslaw *et al.*[9]) and were considered to have received an antigenic stimulus; 15 of the 19 showed a significant rise in *P. tularensis* agglutinins (Saslaw and Carhart[11]). Eight of 14 volunteers immunized with Foshay vaccine exhibited clinical evidence of infection after respiratory challenge (Saslaw *et al.*[10]). Only one showed a rise in brucella agglutinin

TABLE 4.—RELATIONSHIP BETWEEN PEAK *P. TULARENSIS* AND *BR. ABORTUS* AGGLUTININ TITERS IN HUMAN VOLUNTEERS AFTER VACCINATION OR CHALLENGE, OR BOTH, WITH *P. TULARENSIS*

P. tularensis Agglutinin Titer	*Br. abortus* Agglutinin Titer							Total with *Br. abortus* Agglutinins	Total Subjects
	0	*10*	*20*	*40*	*80*	*160*	*320*		
40	4							0	4
80	6		2					2	8
160	13		2					2	15
320	16		5		2	1		8	24
640	14		1	4	2	1		8	22
1280	10	1	1	4				6	16
2560	6	1			1		1	3	9
Totals	69	2	11	8	5	2	1	29	98

titer, whereas agglutinins for *P. tularensis* increased in 3[11]. Similarly, respiratory challenge of volunteers who previously received viable vaccine did not alter brucella antibody titers. Three in this group became ill and one exhibited a rise in tularemia agglutinins[11]. Thus, 30 vaccinated subjects were known to have received an additional antigenic stimulus following intracutaneous or respiratory challenge as measured by development of symptoms or local lesions or increase in tularemia titer. Only 1, however, exhibited an increase in agglutinins to *Br. abortus* as compared to 19 of 30 showing signicant increase in tularemia agglutinins.

No correlation was noted between the titer of the tularemia agglutinins and the incidence of brucella antibodies in the 98 subjects included in the present study. Table 4 shows, for example, that 16 individuals who exhibited agglutinins for *P. tularensis* in high titer (1:1280 and 1:2560) failed to show brucella agglutinins. In all of the 29 who demonstrated antibodies to brucella, the homologous *P. tularensis* titer was always higher. Only one (Subject No. 1, Table 3) exhibited agglutinins to both antigens at dilutions sufficiently similar to have possibly created a diagnostic problem had this individual been suspected of having either brucellosis or tularemia. Only 8 subjects (Table 4) showed brucella antibodies in titers of 1:80 or above while 21 had titers varying from 1:10 to 1:40.

No clear-cut correlation was noted between development of brucella agglutinins and presence of these antibodies before vaccination or challenge. Sixteen of the 98 subjects showed low (1:10 and 1:20) baseline brucella titers whereas 82 were completely negative. Seven of the 16 (44%) and 22 of the 82 (27%) subsequently showed significant rise in agglutinins for *Br. abortus* after vaccination or challenge.

All 15 rabbits immunized with formalin-killed suspensions of 5 strains of *P. tularensis* exhibited comparable peak antitularemia antibody titers (1:640 or 1:1280). No strain differences in antigenicity for rabbits were observed. A total of 14 of 15 also exhibited cross-reacting brucella antibodies after the first antigenic stimulus, although in lower titers (1:20 to 1:320) than the homologous tularemia antibody titers. The remaining rabbit showed brucella antibodies only after a second booster injection at 11 weeks. Curves of antibody production and decline (Fig. 1), as measured by *P. tularensis* bacterial agglutination, polysaccharide hemagglutination, and *Br. abortus* bacterial

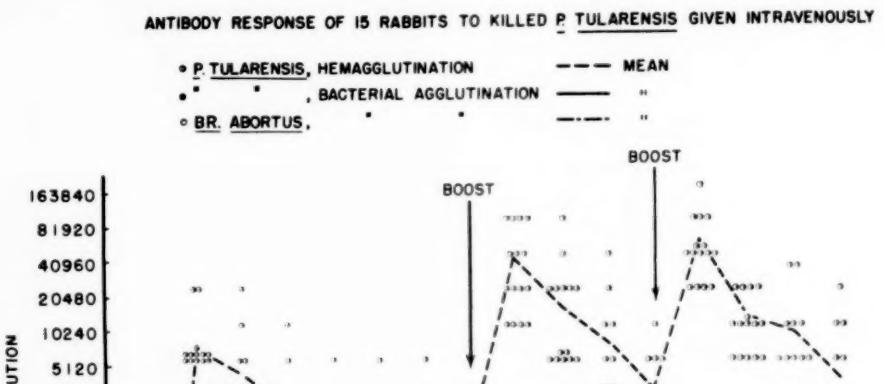

Fig. 1

agglutination, were similar with the exception that booster injections appeared to have a greater effect on polysaccharide hemagglutination titers.

Discussion. These studies confirm, under controlled conditions, that both tularemia vaccination and infection in humans may stimulate the formation of antibodies against *Br. abortus*. Administration of Foshay phenol-killed vaccine resulted in brucella antibody production in 16 of 43 (37.2%) volunteers. These results are in agreement with those of Kadull *et al.*[7] who detected agglutinins for *Br. abortus* in 114 of 278 (41.0%) vaccinated subjects. Six of 29 (20.7%) volunteers in our study who received viable vaccine exhibited brucella antibodies. This corresponded closely to the 23.1% incidence of cross reaction (6 of 26) following infection of nonvaccinated volunteers by either intracutaneous or respiratory routes; after intracutaneous challenge 3 of 12 and after respiratory challenge 3 of 14 showed brucella antibodies. All 6 exhibited frank symptoms of tularemic infection and produced agglutinins for

P. tularensis (Saslaw *et al.*[9-11]). Other workers have demonstrated brucella antibodies in 37.0% (Francis[5]), 22.7% (Francis and Evans[6]), and 18.2% (Stanfield, Taylor and Morgan[13]) of cases of naturally-acquired tularemia.

In the present study, 30 of 51 vaccinated volunteers who were challenged by either intracutaneous or respiratory routes were considered to have received an adequate antigenic stimulus since either symptoms of tularemia or a rise in *P. tularensis* agglutinin titers were observed. Only one of 30 showed a rise in brucella antibodies as compared to 19 of 30 exhibiting a significant rise in tularemia agglutinins. This suggests that the second contact with the tularemia antigen had less effect on heterologous antibody formation than did either vaccination or infection of nonvaccinated men.

All of 15 rabbits immunized with killed *P. tularensis* produced agglutinins for *Br. abortus*. Other workers have shown that rabbits (Poston and Smith[8]) and goats and horses (Foshay[4]) uniformly exhibit brucella agglutinins after inoculation with killed tularemia organisms. Thus, experimental animals and humans appear to react differently in this respect, although it must be admitted that in our study, the 15 rabbits received a much larger antigenic stimulus, on a body weight basis, than did the volunteers. Thus in the interpretation of serologic data, one must be cognizant of the fact that cross reacting brucella antibodies occur frequently following vaccination with killed Foshay vaccine (37.2%) or viable vaccine (20.7%) or after intracutaneous (25%) or respiratory (21.4%) challenge. This degree of cross-reaction, however, is considerably less than the almost 100% incidence observed following immunization of animals. As with the interpretation of any indirect method, namely, the serologic aid to diagnosis, these data must always be interpreted in terms of the clinical picture.

Summary. 1. Immunization with Foshay killed tularemia vaccine resulted in significant brucella antibody titers in 16 of 43 (37.2%) volunteers.

2. Brucella antibodies were observed in 5 of 28 (17.9%) volunteers after immunization with viable attenuated vaccine.

3. All of 15 rabbits immunized with killed tularemia organisms exhibited antibodies to *Br. abortus*.

4. Intracutaneous challenge with *P. tularensis* resulted in significant brucella titer rises in 3 of 12 (25%) nonvaccinated volunteers; after respiratory challenge 3 of 14 (21.4%) exhibited brucella antibodies.

5. Only one of 30 previously vaccinated volunteers showed significant rise in brucella agglutinins after either intracutaneous or respiratory challenge.

6. In all instances of cross-reaction, the homologous tularemia agglutinin titer was higher than the heterologous *Br. abortus* titer.

REFERENCES

1. **Eisele, C. W.:** Med. Clin. N. America, **31**, 182, 1947.
2. **Feinberg, R. J.,** and **Wright, G. G.:** J. Immunol., **67**, 115, 1951.
3. **Foshay, L.:** Ann. Rev. Microbiol., **4**, 313, 1950.
4. Idem: J. Infect. Dis., **59**, 330, 1936.
5. **Francis, E.:** J. Am. Med. Assn., **91**, 1155, 1928.
6. **Francis, E.,** and **Evans, A. C.:** Pub. Health Rep., **41**, 1273, 1926.
7. **Kadull, P. J., Reames, H. R., Coriell, L. L.,** and **Foshay, L.:** J. Immunol., **65**, 425, 1950.
8. **Poston, M. A.,** and **Smith, D. T.:** Bact. Proc., 80, 1951.
9. **Saslaw, S., Eigelsbach, H. T., Wilson, H. E., Prior, J. A.,** and **Carhart, S.:** Arch. Int. Med., **107**, 689, 1961.

10. **Saslaw, S., Eigelsbach, H. T., Prior, J. A., Wilson, H. E.,** and **Carhart, S.:** Ibid., **107,** 702, 1961.
11. **Saslaw, S.,** and **Carhart, S.:** Am. J. Med. Sci., **241,** 689, 1961.
12. **Spink, W. W., McCullough, N. B., Hutchings, L. M.,** and **Mingle, C. K.:** Am. J. Clin. Path., **24,** 496, 1954.
13. **Stanfield, C. A., Taylor, P. W.,** and **Morgan, H. R.:** Ibid., **22,** 211, 1952.

SUMMARIO IN INTERLINGUA

IV. Agglutininas a Brucella in Vaccinate e Non-Vaccinate Voluntarios Provocate con *Pasteurella tularensis*

1. Immunisation con vaccino de tularemia, occidite secundo Foshay, resultava in significative titros de anticorpore a brucella in 16 ex 43 voluntarios (37,2%).

2. Anticorpore a brucella esseva observate in 5 ex 28 voluntarios (17,9%) post immunisation con vaccino viabile attenuate.

3. Omne le 15 conilios immunisate con occidite organismos de tularemia exhibiva anticorpore a *Brucella abortus.*

4. Le provocation intracutanee con *Pasteurella tularensis* resultava in significative augmentos del titros de anticorpore a brucella in 3 ex 12 non-vaccinate voluntarios (25%). Post provocation respiratori, 3 ex 14 voluntarios (21,4%) exhibiva anticorpore a brucella.

5. Solmente un ex 30 previemente vaccinate voluntarios monstrava un significative augmento del agglutininas de brucella post provocation, sia intracutanee sia respiratori.

SUMMARIO IN INTERLINGUA

(See page 201 for original article)

Un Studio a Duple Anonymato de Diethylpropiona in Obesitate

Es reportate un studio a duple anonymato de diethylpropiona in 37 patientes in un clinica pro obesitate. Le perdita medie de peso per septimana esseva 1,1 libras in 28 patientes tractate con le droga active e 0,2 libra in 29 patientes recipiente le placebo. Inter le 19 patientes tractate con solmente un del medicationes, le perdita medie de peso per septimana esseva 1,8 libras in le gruppo a diethylpropiona e 0,7 libra in le gruppo a placebo.

Inter le patientes del serie total, transiente e minor effectos adverse occurreva in 18% del patientes recipiente diethylpropiona e in 14% del patientes recipiente le placebo. Nulle major effectos adverse occurreva in ulle del gruppos.

Le valor del technica a duple anonymato in le evalutation de drogas esseva de novo confirmate.

PERICARDIAL EFFUSION IN CLINICALLY INACTIVE COMPENSATED RHEUMATIC HEART DISEASE

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Chronic pericardial effusion may be entirely asymptomatic and unrecognizable by conventional cardiovascular studies. Even massive pericardial effusion may be present without signs of cardiac tamponade. Chronic pericardial and frequently asymptomatic effusions have been reported in persons with myxedema (Freeman[14], Kern *et al.*[17]), anemia (Soloff and Bello[22]), polycythemia (Genecin[15], Soloff and Bello[22]), infections particularly tuberculosis (Alexander[1], Barker and Johnston[2] Freedman[13], Soloff and Zatuchni[23]), so-called collagen diseases particularly lupus erythematosus (Bergen[3], Meltzer[18]), neoplasms (Tucker[26]), after radiation (Blumenfeld and Thomas[4]) and in those with chylous obstruction (Groves and Effler[16]). Chronic pericardial effusion of unknown origin was first reported by Barker and Johnston[2], and subsequently, by Contro, DeGiuli and Ragazzini[10], by Connolly *et al.*[9] and also by Scheurer[20]. It is noteworthy that most of their patients had electrocardiographic evidence of P mitrale and unstable rhythms characterized by recurrent atrial fibrillation and flutter.

Pericardial effusion in congestive heart failure is common in persons with rheumatic heart disease but rare in those with other forms of heart disease. Furthermore, we have been surprised on several occasions to find pericardial effusion at operation in rheumatic patients who had no clinical evidence of either rheumatic activity or congestive heart failure (Soloff[21]).

This study attempts to determine the minimal incidence of pericardial effusion in persons with clinically inactive compensated rheumatic heart disease.

Materials and Methods. Two hundred and seven patients were selected for study. All had heart disease but were free of congestive failure or clinical evidence of rheumatic activity at the time of study. All had venous angiocardiograms performed in conjunction with diagnostic right heart catheterizations. One hundred and forty-one patients had rheumatic heart disease and 66 had other forms of heart disease. The technique and method of studying venous angiocardiograms have been described elsewhere (Soloff and Zatuchni[23]). Routine chest films for cardiac evaluation before and after angiocardiographic study were reviewed for re-evaluation of the presence of pericardial effusion discovered by angiocardiography. Hemodynamic studies were also reviewed for clues to suggest or establish the presence of effusion.

Pericardial effusion was diagnosed by the demonstration of homogenous density external to the opacified heart usually most prominent on the right side. The homogeneous density may surround the opacified heart or apparently be confined to one region most commonly that bordering on the right atrium. The intrapericardial density is distinctly fainter than that of the opacified heart. Differentiation from other lesions producing extracardiac densities are described elsewhere (Soloff and Zatuchni[23]).

Results. Venous angiocardiography clearly uncovered pericardial effusion

in 5 (3.5%) patients with rheumatic heart disease (Table 1). All were females, 47 to 58 years of age. All had atrial fibrillation. The known duration of heart disease ranged from 1 to 50 years. Although none had clinical evidence of heart failure, a slight elevation of the mean right atrial and of the right ventricular diastolic pressures in C.M. and E.L. raise the possibility that minimal failure may have been present at the time of catheterization. Three patients, E.L., L.T. and M.C. had a history of congestive failure in the past. In each instance, abnormal intrapericardial density external to the opacified right atrium as seen in the posterior anterior films was diagnostic of pericardial effusion.

TABLE 1

Patient	*Age*	*Sex*	*Known duration of heart disease Years*	*Diagnosis*	*Cardiac Catheterization*					
					*RA**	*RV*	*PA*	*PCP**	*C.O.*	*Rhythm*
M.C.	47	F	1	MS,MI	4	29/3	26/14	14	2.3	AF
L.T.	56	F	30	MI	1.5	25/1	26/9	5–8	2.0	AF
W.B.	55	F	?	MS	5	35/4	39/15	5	6.6	AF
C.M.	58	F	6	MS	9	43/10	39/24	24	4.7	AF
E.L.	50	F	7	MS,MI	7	95/9	104/25	17	—	AF

*Mean Pressure

Two patients deserve special comment. C.M. developed clinical and laboratory evidence of hyperthyroidism and was given a therapeutic dose of I_{131} two years before the angiocardiographic study. She then developed clinical evidence of hypothyroidism with a basal metabolic rate of —32%. This disorder may be associated with pericardial effusion. However, there had been no change in cardiac findings and particularly no change in the cardiac silhouette during this 2-year interval.

The other, E.L., presented a very unusual picture. Her 7-year illness was characterized by recurrent and intermittent arthralgia, arthritis, dyspnea, orthopnea, anorexia, peripheral edema, fever and arrhythmia. Repeatedly, Roentgen-ray studies revealed marked cardiac enlargement which was interpreted elsewhere as due to massive cardiac enlargement secondary to rheumatic multivalvular heart disease. Cardiac surgery for valvular heart disease had been refused elsewhere because the heart was regarded as irreversibly massively enlarged due to myocardial disease. She was referred to one of us (L.A.S.) who suspected pericardial effusion that was proved by angiocardiography. Actually the cardiac size was almost normal and myocardial function, as revealed by angiocardiographic circulation times, was relatively good. The cardiac silhouette slowly decreased in size without change in symptoms. Biopsy then revealed an adherent pericardium 2 mm. to 3 mm. thick and free of calcium.

All 66 patients with congenital, hypertensive and arteriosclerotic heart disease were free of angiocardiographic evidence of pericardial effusion.

Discussion. Symptoms and signs of pericardial effusion depend not only

upon the nature and the amount of the effusion but also upon the rapidity of the accumulation of effusion. Thus a rapid accumulation of 200 to 300 ml. of fluid can produce disabling cardiac tamponade whereas much greater amounts of slowly accumulating fluid may produce no symptoms and even no elevation in the venous pressure. Diagnosis of such effusion will depend primarily upon roentgenographic characteristics which are not pathognomonic because conventional roentgenography cannot separate the pericardium from myocardium. Intravenous angiocardiography and the simpler but less diagnostic intravenous carbon dioxide injections (Stauffer *et al.*[24]) are available methods of establishing a definitive diagnosis of pericardial effusion.

Burch and Ray have shown in the experimental animal that the normal pericardium can transfer enormous quantities of salt and water[6]. This property is probably due to the large absorptive surface area of the pericardium and the vigorous action of the heart. Accumulation of fluid within the pericardium, therefore, suggests the presence of pericardial disease which interferes with its absorptive power. Indeed pericardial effusion is most commonly (if not exclusively) found in the presence of structural pericardial disease. In anemia and polycythemia, structural abnormalities may be due to excessive infiltration of the myocardium and pericardium with hemosiderin. The cause of pericardial effusion when found in congenital heart disease without congestive heart failure is not clear, but it is noteworthy that rheumatic heart disease is not rarely associated with congenital heart disease (Boone and Rosemond[5]).

Rheumatic fever which affects the heart is a pancarditic lesion which involves the pericardium. It is not surprising, therefore, that microscopic evidence of pericarditis is found in nearly all instances of rheumatic carditis[8]. It is especially noteworthy that pericardial effusion with congestive failure present at necropsy occurs most commonly in those with rheumatic heart disease (Camp and White[7]).

Our studies indicate that over half of all patients with rheumatic heart disease and congestive failure have pericardial effusion. The present study suggests that pericardial effusion may persist in the absence of failure perhaps due to residual pericardial disease which interferes with normal reabsorptive processes of the pericardium. Thus 4 of our patients with pericardial effusion had failure at some time prior to the time of the angiocardiographic study. Cardiac catheterization was of no diagnostic help. The low cardiac output in patients M.C. and L.T. is commonly encountered with mitral insufficiency with or without pericardial effusions. The intracardiac circulation times were prolonged in all 5 patients, as is frequently the case in the absence as well as presence of pericardial effusion. The elevated right atrial and diastolic right ventricular pressures in E.L. and C.M. may represent a minimal degree of right ventricular failure rather than pericardial effusion. However, it has been estimated that an increase in pericardial fluid of 80 to 100 ml. may be sufficient to raise venous pressures in humans (Stewart, Crane and Deitrick[25]). If so, the effusions in both of these patients, which certainly exceed this degree, may well account for these elevated pressures.

Finally, such effusions may disappear with the development of adhesive pericarditis, changes that have been reported in those with presumably chronic idiopathic effusion (Scheurer[20]).

Summary. 1. Five of 141 patients

with inactive compensated rheumatic heart disease demonstrated pericardial effusion by the technique of venous angiocardiography.

2. An additional 66 patients with congenital, hypertensive and coronary artery disease were free of pericardial effusion as judged by this technique.

3. It is suggested that rheumatic pericarditis may alter the reabsorptive capacity of the pericardium so that fluid accumulated during a bout of activity or failure may persist for prolonged periods of time so that when it is discovered, its cause may be overlooked.

REFERENCES

1. **Alexander, J. S.:** Brit. Med. J., **2**, 463, 1919.
2. **Barker, D. S.,** and **Johnston, F. D.:** Circulation, **2**, 134, 1950.
3. **Bergen, S. S., Jr.:** Ibid., **22**, 144, 1960.
4. **Blumenfeld, H.,** and **Thomas, S. F.:** Radiology, **44**, 335, 1945.
5. **Boone, J. A.,** and **Rosemond, H. M.:** Am. J. Med., **28**, 247, 1960.
6. **Burch, G. E.,** and **Ray, C. T.:** Circ. Res., **6**, 755, 1958.
7. **Camp, P. D.,** and **White, P. D.:** Am. J. Med. Sci., **184**, 782, 1932.
8. Clinicopathologic Conference: Am. J. Med., **25**, 267, 1958.
9. **Connolly, D. C., Dry, T. J., Good, C. A., Clagget, O. T.,** and **Burchell, H. B.:** Circulation, **20**, 1095, 1959.
10. **Contro, S., DeGiuli, G.,** and **Ragazzini, F.:** Ibid., **11**, 844, 1955.
11. **Daniel, G.,** and **Puder, S.:** Virch. Arch. Path. Anat., **284**, 853, 1932.
12. **Eelman, P.:** Brit. Heart J., **7**, 147, 1945.
13. **Freedman, E.:** Am. J. Roent. & Rad. Therapy, **42**, 38, 1939.
14. **Freeman, E. B.:** Ann. Int. Med., **7**, 1070, 1934.
15. **Genecin, A.:** Am. J. Med., **26**, 496, 1959.
16. **Groves, J. K.,** and **Effler, D. B.:** New England J. Med., **250**, 520, 1954.
17. **Kern, R. A., Soloff, L. A., Snape, W. J.,** and **Bello, C. T.:** Am. J. Med. Sci., **217**, 609, 1949.
18. **Meltzer, J. I.:** Am. J. Med., **20**, 638, 1956.
19. **Merrill, A. J.:** Am. Heart J., **16**, 505, 1938.
20. **Scheurer, J.:** Circulation, **21**, 41, 1960.
21. **Soloff, L. A.:** Personal observation.
22. **Soloff, L. A.,** and **Bello, C. T.:** Circulation, in press.
23. **Soloff, L. A.,** and **Zatuchni, J.:** Am. J. Med. Sci., **234**, 687, 1957.
24. **Stauffer, H. M., Soloff, L. A., Zatuchni, J.,** and **Carter, B.:** J. Am. Med. Assn., **172**, 1122, 1960.
25. **Stewart, H. J., Crane, N. F.,** and **Deitrick, J. E.:** Am. Heart J., **16**, 189, 1938.
26. **Tucker, P.:** Ztschr. f. klin. Med., **139**, 208, 1941.

SUMMARIO IN INTERLINGUA

Effusion Pericardial in Clinicamente Inactive, Compensate Morbo Cardiac Rheumatic

1. Cinque inter 141 patientes con inactive, compensate morbo cardiac rheumatic monstrava effusion pericardial in examines per medio del technica de angiocardiographia venose.

2. Un gruppo additional de 66 patientes con congenite, hypertensive, e arteriosclerotic morbo cardiac esseva libere de effusion pericardial, a judicar per le mesme technica.

3. Es suggestionate que pericarditis rheumatic es possibilemente capace a alterar le capacitate del pericardio pro reabsorption, de maniera que liquido accumulate durante un accesso de activitate o in disfallimento pote persister durante longe periodos de tempore, con le resultato que quando illo es discoperite, su causa escappa facilemente al observator.

ANIMAL EXPERIENCE WITH THE CALCIUM-DIGITALIS TOLERANCE TEST

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It has been shown that calcium alone can produce, when given intravenously to animals, the electrocardiographic changes seen with digitalis and with digitalis toxicity, and data are available to show that it takes less calcium to produce these changes when the animal has been given digitalis, and *vice versa* (Golden and Brams[3], Hoff, Smith and Winkler[5,12]). A calcium-digitalis tolerance test has been used in humans to assess their level of digitalization (Nalbandian, Gordon and Kaufman[9]). This test is based on the studies which have demonstrated the synergism between calcium and digitalis in both the isolated heart and in the intact experimental animal (Baker and Baker[1], Gold and Kwit[2], Salter, Sciarini and Gemmel[10], Salter and Runnels[11]). Except for the original work, references in the literature to this test are infrequent. Lown, Marcus and Levine have reported that, "Our studies fail to corroborate its value"[6].

We had originally designed an experiment using the calcium-digitalis test to follow digitalis levels in animals before and after heart-lung by-pass. However, because of inconstant results in the control animals we were led to further study the test *per se*.

Methods. The calcium-digitalis tolerance tests were done on 10 mongrel dogs; 8 of these animals were intact for the entire series of tests, while 2 had thoracotomy, heart-lung by-pass, and intracardiac procedures prior to the third of the series of tests on that animal. The animals were anesthetized with 2% sodium thiopental, and kept lightly anesthetized during the procedure with additional doses as needed. Endotracheal cannulization was not needed. Respiratory depression was not seen. Immediately following anesthesia the animal was weighed, the electrocardiograph electrodes put in place via needles in the subcutaneous spaces of each limb, and the electrocardiogram was connected so that continuous visualization of the tracing was available directly, or through the use of a Viso-scope. Standard limb leads and augmented leads were obtained on each animal prior to commencement of the tolerance test. Usually, standard limb lead II was monitored during the performance of the test. Besides visual monitoring of the tracing, actual recording was also done at one and 2 minutes following each calcium test dose, and more often as indicated. The calcium-digitalis tolerance test was performed as described by Nalbandian,

TABLE 1.—COMPILATION OF DATA OBTAINED FROM 10 ANIMALS AND 24 TESTS. TESTS LABELED "DIGITALIS CONTROL" ARE THOSE DONE AFTER THE ANIMAL HAD RECEIVED DIGITOXIN. WHEN A 2ND DIGITALIS CONTROL TEST IS INCLUDED IT WAS DONE 4 HOURS AFTER THE ORIGINAL DIGITALIS CONTROL. EDTA REFERS TO ETHYLENEDIAMINETETRACETIC ACID

CALCIUM-DIGITALIS TOLERANCE TEST RESULTS

Dog and Test	Kg. wt.	I.V. Digitoxin mg./kg.	Calcium Tolerance test positive with dose no.	Endpoint	Comment	Sinus Arrhythmia
1 Control	24.1	.04	None	PVC'S	None	Dose 6
Digitalis control			7	PVC'S		
2 Control	20	.04	10	Bigeminy	Arrhythmia p̄ EDTA	Dose 2-8
Digitalis control			6	Bigeminy	Retched on dose 9	Dose 2-6
Digitalis control			7	Bigeminy		
3 Control	27.5	.06	10	PVC'S		Dose 5-7
Digitalis control			4	Bigeminy	Arrhythmia p̄ EDTA	Dose 1-3
4 Control	23	.06	9	PVC'S	Arrhythmia p̄ EDTA (2 doses)	Dose 4-7
Digitalis control			10	Bigeminy	Arrhythmia p̄ EDTA	Dose 3-8
Digitalis control			11	Bigeminy	Arrhythmia p̄ EDTA	Dose 9-10
5 Control	22.5	.08	8	Bigeminy	2 doses EDTA to = decrease not abolishment PVC'S	Dose 3-11
Digitalis control			7	Bigeminy	2 doses EDTA still PVC'S for 3 hrs.	Dose 1-6
Digitalis control			1	Bigeminy	occas. PVC ā dose 1	
6 Control	22	.06	None		None	
Digitalis control			None			
Digitalis control			None			
7 Control	12	.08	None		None	Dose 3-5
Digitalis control			5	Nodal Rhythm		Dose 3-4
8 Control	17	.07	9	Bigeminy	Retched on dose 9	
Digitalis control			None		Retched on dose 9	
9 Control	18	.08	11	2°AV Block	Retched on dose 12	Dose 1-7
Digitalis control			8	2°AV Block		Dose 1-7
10 Control	23	.08	2	Bigeminy	Bigeminy 10′ p̄ EDTA	
Digitalis control			6	PVC'S		Dose 2-6

with graduated doses of 10% calcium gluconate (USP) given to the animal intravenously every 2½ minutes until an electrocardiographic endpoint indicating digitalis toxicity had occurred, or 12 doses had been given. Following the dose producing the positive response, the amount of 10% or 20% disodium ethylenediaminetetracetic acid (disodium EDTA)* was given that would combine with one-half the previous calcium dose, as per Nalbandian *et al.*[8].

Using each animal as his own control, the test was first done in the undigitalized state. Then, while the animal was still anesthetized, an intravenous dose of digitoxin (USP) in an amount between 0.04 and 0.09 mg. per kg. of body weight was given. The animal was then returned to its quarters to recover.

*Disodium ethylenediaminetetracetic acid, Versenate trademark, was furnished through the courtesy of the Riker Laboratories, Inc., Northridge, California.

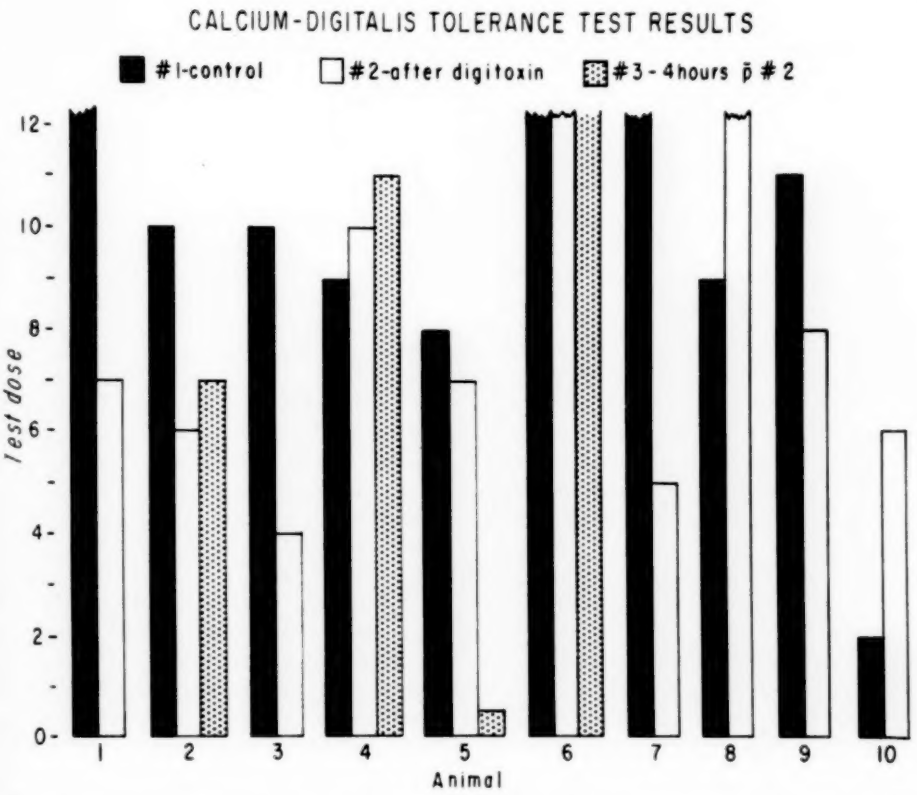

Fig. 1.—Graphic representation shows results of calcium tolerance test before and after digitoxin administration.

Sixteen to 22 hours later the animal was again anesthetized with 2% sodium thiopental and a second calcium-digitalis tolerance test was done. In 2 animals the digitalis-calcium tolerance test was repeated 4 hours following heart-lung by-pass and intracardiac surgery. In 4 other animals the test was repeated 4 hours later, but with the animal remaining intact during that period of time.

Results. Table 1 provides a summary of the changes occurring in the animals. The salient findings are that of 10 dogs tested, 7 animals had a positive calcium tolerance test in the control state; that is, prior to receiving any digitalis. This occurred after dose 8 in all of the animals except No. 1, in which it occurred with the second dose. Except for one animal in which a second degree atrioventricular block was noted, all of the animals who had a positive test had premature ventricular contractions as the manifestation of toxicity.

Following the administration of digitoxin, the "second" calcium-digitalis tolerance test showed that 6 of the animals (No. 1, 2, 3, 5, 7 and 9) had a test which was positive on a smaller dose than in the control state. However, 3 of the animals (No. 4, 8 and 10) had a test which was positive at a greater dose than was found to produce a positive test in the control state; and one animal (No. 6) had neither a positive test in the control state nor in the post-digitoxin state (Fig. 1).

In almost all of the tests a sinus

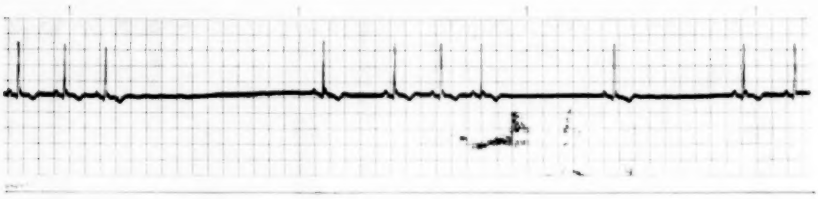

Fig. 2.—This shows the magnitude of the sinus arrhythmia commonly seen during the calcium tolerance test. This particular arrhythmia occurred in dog No. 3 in the control (undigitalized) state one minute after calcium dose No. 9.

arrhythmia occurred on the second or third dose of calcium. This arrhythmia became marked in most of the animals during the early part of the tolerance test (Fig. 2). Also, in 4 of 10 animals there was retching at some time during the tolerance test, usually during the latter phase.

In 7 of 26 tests done on 10 dogs (and in 5 of 10 dogs) the administration of disodium EDTA as described did not abolish the arrhythmia after a period of 5 minutes, and in some of the animals the arrhythmia recurred following its original abolition with disodium EDTA. In one animal a second dose of disodium EDTA did not abolish the arrhythmia.

Discussion. The synergistic relationship of calcium and digitalis on the myocardium has been well established in the literature (Golden and Brams[3], McGuigan and Higgins[7]). An exact quantitative relation has been shown in the isolated heart preparations suspended in a perfusion media in which variations in both the calcium, potassium and digitalis concentrations were made (Salter and Runnels[11], Smith, Winkler and Hoff[12]). Nalbandian *et al.* have described a calcium-digitalis tolerance test in experimental animals and in humans. From their studies they were able to derive test doses and criteria for determining the level of digitalization of the subject being tested. In their published animal work 3 control dogs were used and in these animals an endpoint was not reached after 12 doses of calcium (the complete test)[8]. This is in direct contrast to our findings in which 7 of 10 animals showed a positive test in the undigitalized state. Their data would indicate that the animals used weighed between 8 and 14 kg., while as noted our animals usually weighed over 20 kg., with only one animal weighing less than 14 kg. (Table 1). This should mean that the blood calcium levels in our animals would be less for any given dose than in the animals of their study. Our animals were anesthetized with sodium thiopental. There is no evidence to indicate that the latter drug has any effect on blood calcium levels, or that it sensitizes the heart to calcium (Goodman and Gilman[4]).

Besides the presence of a significant number of positive tests in the control state, another phenomenon noted rather consistently was sinus arrhythmia. This usually appeared on the second or third dose and by the fifth or sixth dose it was usually marked, with long periods of asystole occurring. Also, as noted previously, retching occurred in a number of animals. In 3, this occurred during the control state without digitalis. This, therefore, seemed directly related to the administration of calcium gluconate.

The findings after the administration of digitoxin of magnitude to produce at

least partial digitalization in every animal were again interesting. Six of 10 dogs did have a test which was positive at a lower dose than during the control study. However, 3 of 10 animals received doses of calcium in excess of that required to produce an electrocardiographic endpoint in the control state. In 2 animals, this was a very significant difference of 3 or 4 doses. These latter 3 animals had received 0.06 to 0.08 mg. per kg. of digitoxin intravenously. The one animal with no endpoint after digitalization had received 0.06 mg. per kg. of digitoxin.

To evaluate the reproducibility of the test a second test was done in 4 animals 4 hours after the first test done in these digitalized animals. Three of these had different endpoints from the preceding test; 2 (no. 2 and 4) had an endpoint one test dose later, while the third (No. 5) had an endpoint 6 doses earlier. This type of "control" suggested instability of the test, as did the results in the animals when the control and post-digitalis control tests were correlated. It is not felt that the interpretation of the electrocardiographic endpoint would account for the findings, since the endpoint was bigeminy (No. 2, 3, 5 and 10), or multifocal premature ventricular contractions (No. 4), or second degree atrioventricular block (No. 9), endpoints incapable of being misinterpreted.

Also brought out was the inability of disodium EDTA to block the endpoint in a significant number of cases. In animal No. 4, bigeminy recurred 33 minutes after the administration of disodium EDTA in one test; and in another test in the same animal, 2 doses did not abolish the arrhythmia. In still another animal (No. 5) the standard dose of disodium EDTA did not show any change in the endpoint. Subsequently a second dose resulted only in a decrease of the premature ventricular contractions. In this animal the bigeminy recurred frequently for 3 hours. Disodium EDTA did not block the arrhythmia within a period of 5 minutes in 50% of the dogs (or in 28% of the tests). Although this was very disturbing, none of the dogs had a serious or fatal arrhythmia. However, one animal did die approximately 48 hours after the second calcium-digitalis tolerance test; that is, the test after the administration of digitoxin.

Summary. Ten mongrel dogs were subjected to the calcium-digitalis tolerance test in the nondigitalized state and after digitoxin administration. Seven dogs had a positive test in the control state, and 3 required more calcium after the administration of digitoxin than in their control state. The results of this test were so variable that we were unable to show a strictly quantitative relation between digitalis and this type of calcium administration. The reason our results differ from those in previous reports is not obvious.

REFERENCES

1. **Baker, W. W.,** and **Baker, J. M.:** Circulation Res., **3,** 274, 1955.
2. **Gold, H.,** and **Kwit, N.:** Science, **86,** 330, 1937.
3. **Golden, J. S.,** and **Brams, W. A.:** Ann. Int. Med., **11,** 1084, 1938.
4. **Goodman, L. S.,** and **Gilman, A.:** Pharmacological Basis of Therapeutics. New York: Macmillan Co., 1955.
5. **Hoff, H. E., Smith, P. K.,** and **Winkler, A. W.:** Am. J. Physiol., **125,** 162, 1939.
6. **Lown, B., Marcus, F.,** and **Levine, H. O.:** New England J. Med., **260,** 301, 1959.
7. **McGuigan, R. A.,** and **Higgins, J. A.:** J. Lab. & Clin. Med., **23,** 839, 1937-38.
8. **Nalbandian, R. M., Gordon, S., Campbell, R.,** and **Kaufman, J.:** Am. J. Med. Sci., **233,** 503, 1957.

9. **Naibandian, R. M., Gordon, S.,** and **Kaufman, J.:** Ibid., **234,** 391, 1957.
10. **Salter, W. T., Sciarini, L. J.,** and **Gemmel, J.:** J. Pharmacol. & Exper. Therap., **96,** 372, 1949.
11. **Salter, W. T.,** and **Runnels, E. A.:** Am. J. Physiol., **165,** 520, 1951.
12. **Smith, P. K., Winkler, A. W.,** and **Hoff, H. E.:** Arch. Int. Med., **64,** 322, 1939.

SUMMARIO IN INTERLINGUA

Experientias in Animales con le Test de Tolerantia pro Calcium e Digitalis

Dece canes bastarde esseva subjicite al test de tolerantia pro calcium e digitalis in le stato non-digitalisate e post le administration de digitoxina. Septe canes habeva un test positive in le stato de controlo, e 3 requireva plus calcium post le administration de digitoxina que in le stato de controlo. Le resultatos del test esseva si variabile que nos non poteva demonstrar un relation strictemente quantitative inter digitalis e iste typo de administration de calcium. Le rationes que pote explicar proque nostre resultatos differe ab illos de previe reportos non es obvie.

SUMMARIO IN INTERLINGUA

(See page 183 for original article)

Absentia de Reflexos Profunde: Un Indicio Diagnostic de Non-Suspicite Diabete

1. Es presentate casos representative pro indicar le signification clinic del absentia del reflexos profunde como indicio in le diagnose de diabete in le absentia de hyperglycemia e glycosuria.

2. Le application practic de iste constatation deberea esser de grande adjuta in le effortio de solver certe obscur problemas clinic.

3. Le implicationes theoric del constatation que diabete pote presentar se sin patente deficit in le metabolismo de hydratos de carbon es de importantia fundamental. Illos suggere que diabete es un generalisate processo pathologic; que su numerose aspectos pote occurrer in independentia de un disordine in le economia de hydratos de carbon; e que le varie faciettas del morbo es phenomenos concomitante plus tosto que complicationes.

ABSENT DEEP REFLEXES: A DIAGNOSTIC CLUE IN UNSUSPECTED DIABETES

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The most reliable objective criterion in the diagnosis of diabetic neuropathy is the bilateral symmetrical absence of knee jerks and ankle jerks. In a related study it was shown that deep reflexes are normally present, even in old age, thus validating the significance of their absence in diabetic neuropathy (Ellenberg[2]). Absence of deep reflexes therefore has clinical significance, requires explanation and merits investigation.

The present report concerns itself with the application of this fact, namely, that the absence of deep reflexes indicates an abnormal state. In the course of a study of deep reflex responses in a general population some instances were encountered where these reflexes were bilaterally absent and there was no associated overt neurologic disease to explain this finding. Although there was neither hyperglycemia nor glycosuria, the suspicion of diabetes with diabetic neuropathy was entertained since many of these patients had clinical syndromes entirely compatible with the diagnosis of diabetes and its known complications. The original suspicion was enhanced by the commonly present family history of diabetes and corroborated by an elevated 2-hour postprandial blood sugar and a diabetic type of glucose tolerance test.

The following case reports are illustrative examples:

Case Reports. CASE 1. This 52-year-old colored woman was admitted with a history of recurrent episodes of dizziness and fainting. Prior investigation revealed the basis to be Stokes-Adams syndrome consequent to a complete heart-block. There was no polyuria, polydypsia or weight loss. Her past history was negative. There was no previous knowledge of diabetes or hypertension. The family history was negative for diabetes.

On examination the blood pressure was 160/70 mm. Hg; a grade three systolic murmur was heard at the base and apex; a thrill was palpable over the carotid arteries; the peripheral pulses were patent. Neurological examination revealed absent ankle jerks and knee jerks bilaterally; examination of the fundi revealed two pin-point hemorrhages suggestive of diabetic retinopathy. Laboratory findings revealed: urine negative for reducing substances; fasting blood sugar 88 mg. per 100 ml.*; blood urea nitrogen 15 mg. per 100 ml.; cholesterol 280 mg. per 100 ml. Electrocardiogram confirmed the presence of complete heart block with atrioventricular dissociation.

Although the fasting blood sugar was normal and the urine was negative for sugar, the diagnosis of diabetes was entertained in view of the bilateral absence of deep reflexes in the presence of severe coronary arteriosclerosis in a woman. This was confirmed by an elevated 2-hour postprandial blood sugar of 180 mg. per 100 ml. and a typical diabetic glucose tolerance test.

It is interesting to note that in an effort to control the heart block, which at times required the use of the pacemaker, a trial of corticosteroids was undertaken. While on steroids the patient developed frank hyperglycemia and glycosuria.

COMMENT. A clinical picture (severe coronary arteriosclerosis in a woman)

* All blood sugar determinations were made using the Somogyi method. By this method 100 mg. per 100 ml. is the upper limit of fasting blood sugar, and 120 mg. per 100 ml. is the accepted upper limit of the 2-hour postprandial value.

compatible with and explained by diabetes plus the absence of deep reflexes indicative of diabetic neuropathy led to the establishment of the correct underlying etiological diagnosis.

CASE 2. This 73-year-old white woman sustained an intracapsular fracture of the left femur. She was admitted to another hospital, but because of the development of atrial flutter, surgery was postponed and she was transferred to the Mount Sinai Hospital one week later. She stated that for the past 24 hours she had urinated infrequently and only small amounts. Family history and past history were noncontributory.

On examination, she was slightly disoriented. An apical systolic murmur was present; blood pressure was 180/60 mm. Hg; the peripheral pulses were all patent. A large mass was palpable in the abdomen; it was smooth, movable, arose from the pelvis and extended to the umbilicus. Urethral catheterization yielded 1800 ml. of urine and was followed by disappearance of the mass. At no time did the patient complain of urinary discomfort. The blood urea nitrogen which had been 10 mg. per 100 ml. at the time of fracture had now risen to 98 mg. per 100 ml. Twenty-four hours after catheterization the patient had still not voided and at this time a Foley catheter was left indwelling. Urinalysis revealed two plus albuminuria, no sugar and many white blood cells; blood Wassermann was negative; hemoglobin 11.6 gm.%; sedimentation rate 35 mm. per hour; white blood cell count 12,800 per c.mm.

It was obvious that urinary retention was present, but the explanation for this posed a diagnostic problem for which there seemed no ready answer. Careful neurological evaluation revealed an absence of knee jerks and ankle jerks associated with diminished vibratory and pin-prick sensations in the lower extremities. Although the fasting blood sugar was 84 mg. per 100 ml. the neurological signs pointed to the diagnosis of diabetes and a diabetic neurogenic bladder. This was further suggested by a lumbar puncture which revealed the spinal fluid protein to be elevated to 100 mg. per 100 ml. with all other aspects, including Wassermann test, being normal. Therefore, a 2-hour postprandial blood sugar was performed which showed an abnormal elevation to 168 mg. per 100 ml. Cystometrogram revealed complete absence of sensation even after the instillation of 800 ml. of fluid and a pressure of 25 mm. Following the indwelling catheter, the blood urea nitrogen fell to normal levels. Repeat cystometrogram revealed persistence of an atonic bladder without sensation. Cystoscopy revealed a bladder with trabeculation and normal except for increased capacity. Resection of the internal vesical sphincter was therefore advised, but the patient refused and was discharged for chronic care with an indwelling catheter.

COMMENT. The bilateral absence of deep reflexes led to the suspicion of diabetes. The elevated 2-hour postprandial blood sugar, the elevated spinal fluid protein and the hypotonic bladder all indicated a neurogenic bladder on the basis of diabetic neuropathy. Further inquiry revealed that for the past year there had been some frequency of urination and nocturia. The sequence of events would then suggest a previously partial diabetic neurogenic bladder with the bone fracture acting as a stress situation resulting in complete urinary retention (Ellenberg[3]).

CASE 3. This 58-year-old woman was admitted to the Mount Sinai Hospital with a history of vomiting, weakness and dizziness. She was a known mild hypertensive of 10 years' duration during which time she had been observed in the outpatient clinic. For the past year she manifested the classical evidences of a nephrotic syndrome, accompanied by azotemia, weakness, nausea and failing vision. For the past several weeks she had noted an ulcer on the right big toe. Family history revealed that her mother had diabetes mellitus.

On examination, generalized anasarca was present. She was lethargic and there was a uremic odor on the breath; blood pressure 160/100 mm. Hg; fundi showed grade three retinopathy with exudates and hemorrhages; a small effusion was present at the right base; the liver was enlarged two finger breadths below the costal margin; all peripheral pulses were palpable; there was a necrotic ulcer at the tip of the right toe with surrounding cellulitis; neurological examination revealed absence of ankle jerks and vibratory sensation in the toes.

Urinalysis revealed two to four plus albuminuria with many white cells and some casts;

hemoglobin 5 gm.%; sedimentation rate 127 mm. per hr.; blood urea nitrogen 50 mg. per 100 ml.; carbon dioxide 16.5 mEq. per L.; total protein 5.5 gm.% with albumin 2.6 and globulin 2.9; sodium 129 mEq. per L.; potassium 4.2 mEq. per L.; cholesterol 310 mg. per 100 ml.; creatinine 7.6 mg. per 100 ml.; 24-hour Esbach showed 4 gm. of protein per L.

The patient was at first thought to have a nephrotic syndrome secondary to chronic diffuse glomerulonephritis. However, the presence of a nephrotic syndrome in association with the absent ankle jerks strongly raised the question of diabetes mellitus even though the fasting blood sugar was 64 mg. per 100 ml. An oral glucose tolerance test showed a 2-hour value of 133 mg. per 100 ml. The intravenous glucose tolerance test showed a rise at one-half hour to 220 mg. per 100 ml. and at one hour to 191 mg. per 100 ml. These abnormal values validated the diagnosis of diabetes.

The patient was discharged to the outpatient department. In the clinic a fasting blood sugar was elevated to 109 mg. per 100 ml. The patient was readmitted 6 months later because of marked increase in her azotemia with progressive rise of the blood urea nitrogen to 146 mg. per 100 ml. and the creatinine to 17.4 mg. per 100 ml. Examination at this time was the same as on the previous admission except that the knee jerks were absent as well as the ankle jerks. The urine was loaded with Gram-positive cocci and white cells in addition to the persistent four plus albuminuria. A blood culture grew out paracolon bacillus. Although energetically treated, she died in coma shortly after admission.

A necropsy showed the classical findings of Kimmelstiel-Wilson lesion, namely nodular hyalinization of the glomeruli as well as arterio- and arteriolosclerosis.

COMMENT. The presence of a nephrotic syndrome in a woman with a family history of diabetes plus a necrotic ulcer of the toe in the presence of good pulses suggested diabetes. The absence of deep reflexes established the diagnosis under these circumstances. The retinopathy which was originally thought to be on a hypertensive, uremic basis was entirely compatible with diabetic retinopathy. The elevated glucose tolerance test curves were corroborative. The necropsy, of course, is entirely diagnostic. This could easily have become another "unexplained case" of Kimmelstiel-Wilson disease not due to diabetes.

CASE 4. This was the first Mount Sinai Hospital admission of a 52-year-old Puerto Rican woman. Her chief complaints were typical of acute myocardial infarction. This diagnosis was confirmed by classical electrocardiographic changes, fever, elevated sedimentation rate, leukocytosis and increase in serum transaminase. The blood pressure was 130/80 mm. Hg. There was no history of hypertension or diabetes and no family history of diabetes.

Laboratory investigation revealed a fasting blood sugar of 79 mg. per 100 ml.; blood cholesterol 220 mg. per 100 ml.; and blood urea nitrogen 14 mg. per 100 ml. Urinalysis was normal.

The pathogenesis of the coronary sclerosis and myocardial infarction in this middle-aged woman remained unexplained. Subsequently a careful neurological examination was performed which revealed the bilateral absence of knee jerks and ankle jerks. On this basis the diagnosis of diabetes mellitus was made which was confirmed by a 2-hour postprandial blood sugar of 150 mg. per 100 ml. and further documented by a typical diabetic glucose tolerance test.

COMMENT. The presence of myocardial infarction in a female in the absence of hypertension and hypercholesterolemia raised the possibility of diabetes mellitus. In this instance the absence of glycosuria and the presence of a normal fasting blood sugar were initially accepted as eliminating this possibility. However, the demonstration of absent knee jerks and ankle jerks led to the laboratory corroboration of the underlying diabetes and thus revealed the pathogenesis of the basic vascular defect.

CASE 5. This was the first Mount Sinai Hospital admission of a 76-year-old white woman who fell and sustained an intracapsular fracture of the hip. Past history was negative; family history was noncontributory.

On examination, the blood pressure was normal and there were no significant abnormalities other than those relating to the

fracture. Urinalysis revealed 20 to 30 white blood cells, rare clumps, occasional red blood cells per high power field, two plus albuminuria but no glycosuria. Urine culture revealed *B. proteus* and *Aerobacer aerogenes*. Sedimentation rate was 52 mm. per hour; fasting blood sugar 83 mg. per 100 ml.; cholesterol 280 mg. per 100 ml.; blood urea nitrogen 21 mg. per 100 ml. A roentgenogram of the chest failed to reveal any pulmonary infiltration.

Shortly after admission the patient was noted to be febrile. Although at first it was suspected that the elevation of the temperature might be coming from a urinary tract infection, this was discarded in favor of pulmonary pathology because of the presence of some basal rales. Repeated blood cultures were negative but a leukocytosis varying from 10,000 to 15,000 white blood cells per c.mm. was present. The patient was treated empirically with penicillin and Chloromycetin in therapeutic doses for 10 days with no effect. She was then treated with tetracycline and streptomycin again with no effect on the febrile course. In the interim a Lippmann prosthesis was performed. Repeated routine examinations eventually included a neurological status which elicited the absence of deep reflexes in the upper as well as lower extremity. In addition the upper extremity revealed symmetrical atrophy of the interossei muscles and of the thenar and hypothenar eminences. These findings indicated the presence of diabetes with diabetic neuropathy and raised the possibility of an associated neurogenic bladder. The patient was catheterized and a residual of 300 ml. of urine was obtained. The diagnosis of diabetes was established by demonstrating an elevation of the 2-hour postprandial blood sugar and further confirmed by a diabetic glucose tolerance test. The presence of a neurogenic bladder was established by cystometrogram studies which revealed an atonic bladder with no sensation even after the instillation of 800 ml. of fluid. A lumbar puncture revealed normal manometric readings, normal cell count, negative serology but an elevated spinal fluid protein of 84 mg. per 100 ml.

It was now apparent that the patient had diabetes, diabetic neuropathy and an associated atonic bladder on a neurogenic basis with secondary urinary infection. A Foley catheter was introduced and left indwelling. With this relief of the urinary obstruction, the patient's temperature rapidly cleared and she had an excellent clinical response.

COMMENT. The bilateral absence of deep reflexes was the clue to the diagnosis of diabetes, diabetic neuropathy and neurogenic vesical dysfunction. This led to the institution of the necessary and indicated therapy resulting in prompt alleviation of the threatening clinical picture and eventuated in a rapid recovery for this patient.

CASE 6. A 60-year-old white man entered the hospital with the chief complaint of pain in both lower extremities for the preceding 8 months. Past history was significant in that one year before he had had a cerebral vascular accident, with temporary right-sided paresis and aphasia. Eight months ago, apparently unrelated to the stroke, he had noted pain in both thighs, calves and feet, increasing in severity. The pain was almost always nocturnal, being described as a cramp, worse in the supine position, severe enough to prevent sleep and relieved by walking. He was admitted for the possibility of a spinal cord neoplasm. There was no previous history of diabetes and the family history was negative for diabetes.

On examination, the patient was well developed and not acutely ill. Fundi were negative, with no hemorrhages or exudates; the pupils were normal; blood pressure 170/80 mm. Hg; the peripheral pulses were normal. Neurological examination revealed a residual old right hemiparesis with a mild expressive aphasia; there was mild generalized weakness of both lower extremities; all deep tendon reflexes were absent except those of the right upper extremity. Oscillometric readings of the lower extremities were normal.

Laboratory findings were: urine specific gravity 1.022, two plus albumin, an occasional white blood cell without casts and no sugar; hemoglobin 14 gm.%; white blood count 9,000 per c.mm., with a normal differential; erythrocyte sedimentation rate 37 mm. per hour; blood urea nitrogen 7 mg. per 100 ml.; total protein 7.5 gm.%, albumin 4.1, globulin 3.4; cholesterol 234 mg. per 100 ml.; alkaline phosphatase 5.1 King-Armstrong units; acid phosphatase 1.1 units.

Although the history and evidences of clinical diabetes were completely lacking, the bilateral absence of knee jerks and ankle jerks and symptoms referable to this patient's neuropathy were so strongly suggestive that a clinical diagnosis of diabetes was postulated on the basis of these observations in the light

of previous experience. This was confirmed by a 2-hour postprandial blood sugar level of 222 mg. per 100 ml. As further confirmation, a lumbar puncture was performed which was completely negative in all details except for the elevation of the total spinal fluid protein to 62 mg. per 100 ml. On bed-rest and a diabetic diet the symptoms gradually tended to disappear. Follow-up information disclosed that the improvement has continued.

COMMENT. Here again, the clue to the presence of diabetes was the bilateral absence of deep reflexes in the lower extremities, abetted by symptoms that were entirely compatible with diabetic neuropathy. The elevated blood sugars, the elevated spinal fluid protein unaccompanied by any other abnormalities, and the course corroborated the clinical impression.

CASE 7. This was the first Mount Sinai Hospital admission of a 73-year-old white woman complaining of poor circulation of the right foot. Eight months ago she had noted coldness of the right toes which was followed by cracking of the skin and the development of ulcers on the right heel and at the base of the right toe. One week ago she noted bluish discoloration of the right toes. Family history was significant in that a sister had diabetes.

On examination, the blood pressure was 150/70 mm. Hg; bilateral lenticular opacities were present. No pulses were palpable below the femoral artery bilaterally. There was an ulcer on the volar aspect of the base of the right great toe and on the lateral surface of the heel accompanied by edema of the right foot and bluish discoloration of the right toes. The ankle jerks and knee jerks were absent bilaterally.

Although the fasting blood sugar on admission was 96 mg. per 100 ml. and urinalysis was negative for sugar, a diagnosis of diabetes mellitus was made because of the absence of the deep reflexes in the lower extremity. The presence of a family history was contributory. The diagnosis was established by finding an elevation of the 2-hour postprandial blood sugar to 210 mg. per 100 ml. and further confirmed by a diabetic type of glucose tolerance test.

The course of the patient is of interest in that she developed progressive gangrene of the involved foot with secondary infection. The temperature became elevated and simultaneously she developed frank clinical diabetes. Fortunately, the diagnosis had been established by this time and the necessary therapy was instituted forthwith. The involvement of the foot led to a below the knee amputation. Following this procedure all signs of infection disappeared and the fasting blood sugar once again became normal.

COMMENT. This case illustrates the significance of absent deep reflexes in suspecting diabetes in the absence of overt alteration of carbohydrate metabolism. The positive family history plus the compatible clinical picture were suggestive and the bilateral absence of the knee jerks and ankle jerks was confirmatory. Proper laboratory tests established the clinical diagnosis. The knowledge of the presence of diabetes facilitated prompt recognition of the development of frank clinical diabetes and the prompt institution of therapy.

Discussion. The clinical significance of the ability to diagnose diabetes via the clue of absent deep reflexes is apparent. It has proven to be a great aid in unmasking the pathogenesis of otherwise unexplained syndromes; in establishing the proper diagnosis; in permitting anticipation of the potential occurrence of hyperglycemia and glycosuria with the resulting prompt institution of metabolic regulation; and in indicating specific adjunctive therapies which have at times dramatically altered the clinical course. With increasing experience, it has become evident that a common cause of unexplained symmetrical absence of deep reflexes is diabetic neuropathy. Conversely, the presence of unexplained absence of deep reflexes is enough to strongly suggest the diagnosis of diabetes and makes its exclusion mandatory.

The theoretic implications are of great importance. It is increasingly evident that diabetes mellitus is a generalized fundamental disease process

only one aspect of which is a disorder of carbohydrate metabolism. Other aspects of the syndrome include retinopathy (Wagener[8]), nephropathy (Freedman[6]), neuropathy (Ellenberg[4]) arteriosclerosis (Dry and Hines[1]) and the complications of pregnancy (Jackson[7]). This concept carries with it the implication that any or all of these aspects may occur independently of hyperglycemia and glycosuria and, by the same token, may be the presenting clinical manifestation of diabetes. Further it implies that those facets of diabetes mellitus other than aberration of carbohydrate metabolism are not complications of diabetes but rather are integral parts and basic manifestations of the syndrome of diabetes mellitus.

Diabetic neuropathy presenting as the initial clinical manifestation of diabetes has been previously documented (Ellenberg[5]). In the type of case presented in this paper diabetic neuropathy manifested by absent deep reflexes has led to the diagnosis of diabetes in the absence of clinical hyperglycemia and glycosuria. That the absent reflexes are part of the picture of diabetic neuropathy is supported by the finding of an elevated spinal fluid protein with no other associated abnormalities of the spinal fluid. Thus diabetes mellitus may, at least in this situation, be diagnosed long before there is any clinical evidence of deranged carbohydrate metabolism. The importance of the 2-hour postprandial blood sugar or glucose tolerance test, and the unreliability of the fasting blood sugar in this category is evident.

This approach may well lead to a greater appreciation of the widespread occurrence of diabetes mellitus, an awareness of its more subtle clinical presentations, the earlier recognition of the disease and a better understanding of its fundamental complexities.

Summary. 1. Representative cases are presented to indicate the clinical significance of absent deep reflexes as a clue to the diagnosis of diabetes in the absence of hyperglycemia and glycosuria.

2. The practical application of this finding should be of great aid in helping resolve some obscure clinical problems.

3. The theoretical implication of diabetes presenting without overt carbohydrate metabolic deficit is of fundamental importance: it suggests that diabetes is a generalized disease process; that its many aspects may occur independently of the carbohydrate disorder; and that the various facets of the disease are concomitants rather than complications.

REFERENCES

1. **Dry, T. J.,** and **Hines, E. A., Jr.:** Ann. Int. Med., **14,** 1893, 1941.
2. **Ellenberg, M.:** J. Am. Med. Assn., **172,** 468, 1960.
3. Idem: Am. J. Med. Sci., **238,** 418, 1959.
4. Idem: Ann. Int. Med., **52,** 1067, 1960.
5. Idem: Ibid., **49,** 3, 1958.
6. **Freedman, L. R.:** Bull. Johns Hopkins Hosp., **100,** 132, 1957.
7. **Jackson, W. P. U.:** Lancet, **1,** 614, 1957.
8. **Wagener, H. P.:** Proc. Am. Diab. Assn., **5,** 203, 1945.

(Interlingua on page 182)

INTRAVENOUS TOLBUTAMIDE AND CEREBRAL HEMODYNAMICS AND OXYGEN CONSUMPTION*

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Recent reports have attributed to the oral hypoglycemic agent, tolbutamide, a salutory effect upon the symptoms of the parkinsonian syndrome (Gates and Hyman[2]) and of multiple sclerosis (Sawyer[6]). Although the mechanism of action was not defined in these clinical studies, it was speculated that the therapeutic effect might be related to altered carbohydrate metabolism in the central nervous system tissue, presumably apart from changes caused by cerebral carbohydrate deprivation due to hypoglycemia. We considered it worthwhile to determine whether tolbutamide, if indeed capable of producing symptomatic improvement in these neurological disorders, might not do so by altering cerebral hemodynamics or by exerting a demonstrable direct influence upon cerebral oxygen consumption.

Method. Six patients hospitalized at the New England Center Hospital were the subjects of this study; 5 had organic central nervous system disease and one had no evident cerebral dysfunction. In all subjects except one, observations were performed in the fasting state.

Scheinberg and Stead's modification[7] of Kety and Schmidt's procedure[3] for the determination of cerebral blood flow was employed in this study. The oxygen content of the blood was determined by the manometric technique of Van Slyke and Neill[5]. Mean arterial pressure was obtained directly from the femoral artery by means of a damped aneroid manometer (Alman and Fazekas[1]). Blood pH and CO_2 tension were measured on an Instrumentation Laboratories Meter, employing a glass electrode for measurement of pH and a Severinghaus-type electrode for the determination of CO_2 tension. Glucose values were determined by the Nelson-Somogyi procedure[4,8].

Immediately after control data of cerebral hemodynamics and oxygen consumption were obtained, 1 gm. of tolbutamide dissolved in 10 ml. of water was administered intravenously. Arterial and internal jugular venous blood samples were withdrawn simultaneously, 20 minutes after the injection, for analysis of blood constituents. Forty minutes after injection, cerebral hemodynamic and metabolic observations were repeated.

Results. As is indicated in Table 1, the pharmacological effectiveness of the doses of tolbutamide administered is reflected by moderate to marked reduction of arterial blood sugar levels in every case. Nevertheless, no significant changes in cerebral hemodynamics or cerebral oxygen consumption could be demonstrated, nor were there observed appreciable alterations of arte-

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TABLE 1.— TOLBUTAMIDE AND QUANTITATIVE CEREBRAL MEASUREMENTS

	CBF		*CMRO₂*		*CVR*		*Art. CO₂*			*Art. pCO₂*			*Art. pH*			*Art. Gluc.*			*Cer. R.Q.*		
	C	E_{40}	*C*	E_{40}	*C*	E_{40}	*C*	E_{20}	E_{40}	*C*	E_{20}	E_{40}	*C*	E_{20}	E_{40}	*C*	E_{20}	E_{40}	*C*	E_{20}	E_{40}
Multiple sclerosis	74.6	82.3	4.2	4.3	1.6	1.4	44.0	44.0	43.8	37	35	37	7.36	7.38	7.37	81.4	59.8	47.1	0.7	1.0	1.1
Cerebral vasc. disease*	35.4	43.0	2.5	2.5	3.4	3.4	44.1	43.6	45.4	35	30	32	7.42	7.45	7.45	127.1	125.6	99.1	1.0	1.1	0.9
Cerebral vasc. disease	80.7	66.1	4.4	3.9	1.4	1.7	44.8	46.4	46.2	37	37	37	7.40	7.42	7.41	86.3	76.0	61.4	1.0	1.0	0.9
Cerebral degeneration	38.9	47.0	3.3	3.2	2.5	2.0	44.6	49.6	49.2	34	40	38	7.45	7.42	7.45	71.7	57.7	42.9	1.0	0.8	1.0
Normal	62.8	62.5	3.8	4.1	1.8	1.7	43.1	44.3	43.7	37	38	35	7.44	7.42	7.44	80.4	64.0	42.4	1.2	1.0	1.1
Cerebral embolus and aphasia	62.6	67.4	3.7	3.7	2.0	1.7	47.7	50.4	51.3	42	41	40	7.38	7.42	7.43	75.6	69.8	55.2	1.1	1.1	1.1
										MEAN VALUES											
	59.2	61.4	3.6	3.6	2.1	2.0	44.7	46.4	46.6	37	37	36	7.41	7.42	7.42	87.1	73.5	58.2	1.0	1.0	1.0

* No fasting

CBF indicates cerebral blood flow in milliliters per minute per 100 gm. brain; $CMRO_2$, cerebral oxygen consumption in milliliters per minute per 100 gm. brain; CVR, cerebral vascular resistence in millimeters Hg per milliliter of blood per minute per 100 gm. brain; Art. CO_2, arterial carbon dioxide content in volumes %; pCO_2, carbon dioxide tension in mm. Hg; Art. Gluc., arterial glucose level in mg. per 100 ml.; Cer. R.Q., cerebral respiratory quotient (CO_2/O_2); C, control; E_{20}, 20 minutes after tolbutamide; and E_{40}, 40 minutes after tolbutamide.

rial CO_2 content or tension, nor of arterial pH. Calculation of cerebral glucose uptake was attempted but, in view of the small cerebral arteriovenous glucose difference and the number of sources of possible laboratory error involved in the determination, individual results were not considered to be of satisfactory reliability. The mean cerebral glucose uptake (not included in the table) showed no significant change after tolbutamide administration. Despite the reduction of blood sugar (which fell as low as 42 mg. per 100 ml. in the arterial blood), none of the subjects experienced any unusual symptoms, including those of hypoglycemia. No objective evidence of change in neurological status, either improvement or worsening of the underlying disorder, was noted, nor did unrelated neurological signs become apparent.

Discussion. The results of this investigation fail to demonstrate any change in the cerebral functions measured, associated with intravenous tolbutamide administration in doses sufficient to induce significant reduction in blood glucose levels. Although the experimental conditions were not comparable to those under which the reported clinical trials (Gates and Hyman[2], Sawyer[6]) were conducted, nevertheless, certain conclusions can be drawn concerning the effects of tolbutamide upon overall cerebral circulation and carbohydrate metabolism.

While only one of the subjects of this study had multiple sclerosis and none suffered from the parkinsonian syndrome, we consider it highly unlikely that a quantitatively demonstrable cerebral hemodynamic or metabolic response to tolbutamide, mediated through changes in carbohydrate metabolism, would occur in patients having these disorders and not in others; there is no evidence that the cerebral dysfunction is related to disturbed carbohydrate metabolism. For that matter, the stability of cerebral oxygen consumption in our subjects indicates that oxidative energy production was not influenced by tolbutamide; and the R.Q. approximating unity further denotes that carbohydrate oxidation continued to completion even after administration of the drug. Indirectly, therefore, it can be concluded that cerebral glucose utilization was, in fact, unchanged even in the presence of reduced blood sugar levels. The latter again demonstrates the well-known fact that the blood glucose levels at which hypoglycemic symptoms appear may be quite variable (there were no symptoms in our subjects), perhaps because of adequate delivery rates related to high rates of cerebral blood flow, or to superior blood-tissue transport mechanisms in some cases.

Any therapeutic effectiveness of tolbutamide in multiple sclerosis and parkinsonism is unlikely to depend upon alterations in blood pH and CO_2 tension (it is well recognized that changes of this nature may, by altering cell membrane permeability, influence neuronal excitability) for neither these chemical changes nor the cerebral hemodynamic alterations which they cause could be detected in our subjects; and it is hardly reasonable to suppose that they might, nevertheless, be induced in patients with parkinsonism and multiple sclerosis.

The method used in this study may not detect small, localized alterations in cerebral hemodynamics and oxygen utilization. This might explain failure to demonstrate changes in these functions if one supposes a tolbutamide effect to be exerted only upon the basal ganglia, but such a supposition would be untenable if the drug is accepted to produce improvement in multiple sclerosis. In fact, the unpredictable

loci of the widespread lesions in the latter disorder would imply that any beneficial cerebral effect of tolbutamide must necessarily involve the entire cerebrum, so that if such effects were based upon cerebral circulatory or metabolic changes, these should be readily demonstrable.

One must consider the possibility of differing effects of long-term oral administration and of acute intravenous administration of tolbutamide. In our subjects, relatively large doses were given intravenously so that a marked fall in blood sugar resulted. It would appear improbable, in view of the much milder reduction in blood glucose achieved by oral administration, that the latter route, over however long a period of time, might be associated with demonstrable changes in cerebral hemodynamics and metabolism not induced by far more intensive administration of the drug. We have not, to be sure, excluded the possibility that tolbutamide may influence an unidentified enzyme system, producing pharmacologic effects apart from action upon glucose metabolism, blood pH, or CO_2 tension, and not related to changes in cerebral hemodynamics or oxygen consumption.

Summary and Conclusions. In 6 subjects, intravenous tolbutamide in doses sufficient to cause moderate-to-marked blood sugar reduction failed to influence arterial pH or CO_2 tension, or cerebral hemodynamics or oxygen consumption. It is concluded that any beneficial effect of this drug in the parkinsonian syndrome or in multiple sclerosis, as recently reported from clinical studies, is not related to action on the above-noted functions, nor to a direct effect upon the carbohydrate metabolism of cerebral tissues.

REFERENCES

1. **Alman, R. W.,** and **Fazekas, J. F.:** New England J. Med., **248,** 105, 1953.
2. **Gates, E. W.,** and **Hyman, I.:** J. Am. Med. Assn., **172,** 1351, 1960.
3. **Kety, S. S.,** and **Schmidt, C. F.:** J. Clin. Invest., **27,** 476, 1948.
4. **Nelson, N.:** J. Biol. Chem., **153,** 375, 1944.
5. **Peters, J. A.,** and **Van Slyke, D. D.:** Quantitative Clinical Chemistry; Methods. Baltimore: The Williams & Wilkins Co., p. 321, 1932.
6. **Sawyer, G. T.:** J. Am. Med. Assn., **174,** 470, 1960.
7. **Scheinberg, P.,** and **Stead, E. A., Jr.:** J. Clin. Invest., **28,** 1163, 1949.
8. **Somogyi, M.:** J. Biol. Chem., **160,** 62, 1945.

SUMMARIO IN INTERLINGUA

Tolbutamida Intravenose e le Hemodynamica e Consumption de Oxygeno in le Cerebro

In 6 subjectos, le administration intravenose de tolbutamida in doses sufficiente a causar moderate a marcate grados de reduction del sucro sanguinee non influentiava le pH o le tension de CO_2 in le sanguine arterial e non le hemodynamica o le consumption de oxygeno in le cerebro. Es concludite que si iste droga exerce un effecto benefic in syndrome de Parkinson o in sclerosis multiple (como il esseva reportate recentemente a base de studios clinic), ille effecto non es relationate con un action super le supra-mentionate functiones e non con un effecto directe in le metabolismo de hydratos de carbon in le tissu cerebral.

LATENT PANCREATIC EXOCRINE INSUFFICIENCY IN PATIENTS WITH HYPERGLYCEMIA

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We wish to record the results of I_{131} labeled fat absorption studies carried out on a series of 20 hyperglycemic patients in a search for latent pancreatic exocrine insufficiency. Four of the 20 patients studied were considered to have pancreatic endocrine and exocrine insufficiency by the following criteria: 1) pancreatic exocrine insufficiency as evidenced by an abnormal triolein absorption pattern, 2) a normal oleic acid absorption pattern, 3) diabetic type hyperglycemia and 4) correction of the triolein absorptive defect coincident with oral pancreatin replacement therapy.

Materials and Method. The subjects of this study were 20 patients on the medical service of the Veterans Administration Hospital, Pittsburgh, Pennsylvania. All had elevated fasting and 2-hour postprandial blood sugar levels or diabetic type glucose tolerance curves. None was admitted primarily for symptoms referably to the gastrointestinal tract. Standard laboratory procedures were used for serum levels of calcium, phosphorous, amylase (Van Loon, Likins and Segar[10]), lipase (Tietz, Borden and Stepleton[9]), cholesterol, total lipids (de La Huerga, Yesinick and Popper[4]), total protein, albumin and globulin.

After blocking the uptake of I_{131} by the thyroid gland with Lugol's solution, 25 to 50 microcuries of I_{131}-labeled triolein or oleic acid (Squibb) in capsule form were administered with 240 ml. of milk to fasting patients. Venous blood, urine and stool specimens were assayed for radioactivity by conventional methods and the results expressed as percent of the ingested dose as described in Table 1.

Results. All 20 cases studied had glucose intolerance exhibited by diabetic type glucose tolerance tests or elevated fasting blood glucose and 2-hour postprandial glucose levels. Four of the 20 patients revealed combined endocrine and exocrine insufficiency of the pancreas, a fifth case remains a suspect with a borderline 10% fecal excretion of radioactive triolein, as shown in Table 1 and Fig. 1.

Studies of pancreatic function as outlined in Table 2 were within average range in all 20 cases studied. Roentgenographic examinations of the gastrointestinal tract were negative for calcification of the pancreas.

Family history was positive in 1 of the 4 cases of insufficiency for hyperglycemia. No history suggestive of familial pancreatitis was obtained.

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TABLE 1

		I_{131} labeled triolein test				I_{131} labeled oleic acid test			
Case number	*Patient's initials*	*% ingested dose per L. plasma at 4–6 hrs.*	*% ingested dose per L. plasma at 6–8 hrs.*	*% ingested dose in 0–48 hr. urine*	*% ingested dose in 0–48 hr. stool*	*% ingested dose per L. plasma at 4–6 hrs.*	*% ingested dose per L. plasma at 6–8 hrs.*	*% ingested dose in 0–48 hr. urine*	*% ingested dose in 0–48 hr. stool*
1	WEP	2.5	2.2	91	1	—	—	—	—
2	PD	0.1	—	48	32	0.2	0.4	56	6
3	JE	1.6	1.8	44	5	—	—	—	—
4	JP	2.3	2.0	69	17	2.1	2.3	86	3
5	TK	0.2	0.2	3	26	0.0	0.8	13	1
6	RTK	2.7	2.5	68	1	—	—	—	—
7	CL	0.0	0.2	71	1	—	—	—	—
8	HEG	0.0	0.6	71	2	—	—	—	—
9	LWG	4.0	3.1	76	< 1				
10	JMS	0.2	0.6	68	4	—	—	—	—
11	CJD	3.4	2.0	54	15	3.0	2.0	81	8
12	JA	0.1	0.8	59	2	—	—	—	—
13	AN	2.0	1.8	70	10	—	—	—	—
14	LHJ	2.7	1.9	48	2	—	—	—	—
15	HCB	0.8	1.8	66	1	—	—	—	—
16	MM	2.0	1.3	52	0	—	—	—	—
17	CAC	1.7	—	78	1	—	—	—	—
18	AJM	1.8	1.8	52	1	—	—	—	—
19	DOJ	2.9	2.1	83	5	—	—	—	—
20	KCM	2.7	1.9	63	0	—	—	—	—

Normal range: Percent ingested dose (I_{131} labeled triolein) in 0 to 48 hour urine, 44 to 91%. Percent ingested dose (I_{131} labeled triolein) in 0 to 48 hour stool 0.2 to 9.0%.

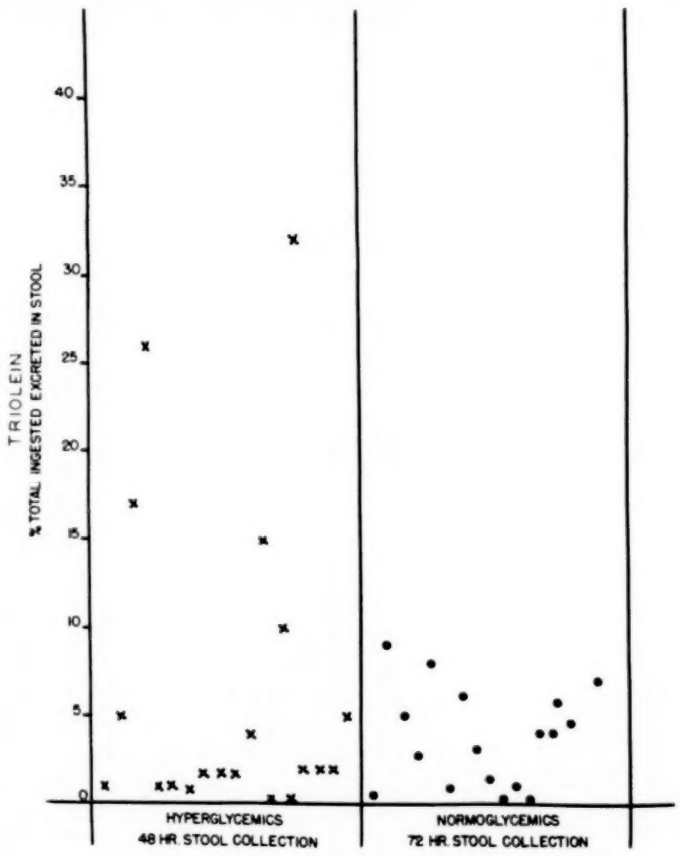

Fig. 1

Case Reports. CASE. 1. J.P., a 32-year-old white man with a familial history of diabetes mellitus was admitted with severe ketonemia. In addition to a history of moderate alcoholic intake, "heartburn" (epigastric) had been present intermittently for the previous 6 months. Several hours prior to admission, he experienced generalized abdominal pain. A history of intermittent episodes of 2 to 3 stools per day, occasionally watery in character was elicited. Barium studies of the entire gastrointestinal tract were negative as was examination of the stool for ova and parasites. No pancreatic calcification was observed by Roentgen-ray. Pancreatic insufficiency was suspected after an abnormal triolein absorption test and confirmed by normal oleic acid absorption.

CASE 2. P.D., a 62-year-old white man had a diagnosis of acute pancreatitis made by biopsy 23 years prior to the current admission for incidental repair of an inguinal hernia when hyperglycemia was discovered for the first time Despite the continued ingestion of alcohol, he had remained free of significant gastrointestinal symptoms, although close questioning revealed intermittent epigastric distress and the occasional passage of 1 to 2 "normal" stools per day. Symptoms referable to the diabetic state were polyuria, polydipsia, and weight loss; this latter may have been due to the pancreatic exocrine insufficiency. Replacement therapy with 5.4 gm. of pancreatin in 3 divided doses converted the 32% fecal excretion of triolein to 6%.

CASE 3. T.K., a 44-year-old Negro male

TABLE 2

Case number	*Patient's initials*	*Age*	*Serum cholesterol (mg./100 ml.)*	*Serum lipids (mg./100 ml.)*	*Serum calcium (mg./100 ml.)*	*Serum phosphorus (mg./100 ml.)*	*Serum lipase (units)*	*Serum amylase (units)*	*Total protein (gm.%)*	*Albumin/globulin ratio*	*Stool trypsin*	*Roentgen ray*	*Duration of hyperglycemic symptoms or diagnosis of diabetes*	*Additional Diagnoses*	*Complications*	*Insulin*	*G. I. Symptoms*	*Family History of Diabetes Mellitus*
1	**WEP**	51	360	380	11.2	4.3	0.4	112	6.1	4.7/1.4	—	—	2M	Obesity. Ketonemia.	None	Lente	None Mod. ethanolism	Positive
2	**PD**	62	210	260	10.9	3.7	1.1	64	8.0	5.0/3.0	Neg	Neg	6W	Pancreatitis, 23 yrs previously.	None	Lente	1–2 stools/day Exc. ethanolism	Negative
3	**JE**	66	165	200	9.8	2.7	0.1	64	6.9	4.5/2.4	—	—	2Y	Granuloma of lung.	None	Lente	None	Negative
4	**JP**	34	280	350	11.0	3.7	0.6	104	6.6	4.5/2.1	Neg	Neg	6M	Ketonemia.	None	Lente	2 stools/day	Positive
5	**TK**	44	300	350	11.8	4.7	0.2	24	5.3	3.1/2.2	—	Neg	6Y	Syphilis. Sickle cell trait. Duodenal ulcer.	Neuropathy	Lente	Gross steatorrhea (5 yrs). Exc. ethanolism	Negative
6	**RTK**	40	360	390	9.8	4.3	0.2	32	5.1	2.3/2.8	—	—	15Y	Traumatic amputation of hand. Nephrotic syndrome	Nephropathy Neuropathy Retinopathy	Lente	None	Negative
7	**CL**	71	284	340	11.4	3.1	0.5	40	7.6	4.3/3.1	—	—	8Y	Arteriosclerotic heart disease. Obesity.	Cataracts	NPH	None Mod. ethanolism	Negative
8	**HEG**	31	310	340	12.2	3.8	0.7	—	6.7	4.6/2.1	—	—	3Y	None	Cataracts	Lente	None	Negative

9	LWG	44	310	420	11.8	3.7	0.6	70	7.1	5.4/1.7	—	Neg	2W	Traumatic ulcers of leg.	None	Lente	None	Negative
10	JMS	63	344	540	11.2	3.5	0.5	120	6.0	4.1/1.9	—	Neg	—	Arteriosclerotic heart disease. Lipid lesion of cornea.	Cataracts	None	None	Negative
11	CJD	48	274	400	9.8	—	0.3	120	7.8	5.4/2.4	—	—	2W	Neurodermatitis.	None	Diabenese	Exc. ethanolism 3–4 stools/day	Negative
12	JA	36	292	520	11.8	3.5	0.3	32	6.4	4.3/2.1	Neg	—	16Y	Unexplained abdominal pain.	Retinopathy Neuropathy	Lente	Abdominal pains. Constipation	Negative
13	AN	47	405	520	9.2	2.6	0.3	60	7.2	4.8/2.4	Neg	—	8Y	Obesity. Arterio-sclerotic heart disease	None	None	2–3 stools/day	Negative
14	LHJ	40	240	350	11.6	4.0	0.4	69	8.4	4.9/3.5	—	—	2Y	Leg ulcers.	None	None	None	Negative
15	HCB	66	290	420	11.4	—	0.6	104	6.0	4.1/1.9	Neg	—	3M	Mycosis fungoides. Arteriosclerotic heart disease.	None	None	None	Negative
16	MM	68	270	380	10.6	3.5	0.7	24	7.3	4.6/2.7	Neg	—	2Y	Obesity. Arterio-sclerotic heart disease with failure.	Cataracts	None	None Mod. ethanolism	Negative
17	CAC	64	244	400	11.2	3.7	0.6	80	6.6	4.2/2.4	Neg	—	4W	Arteriosclerotic heart disease. Anxiety reaction.	None	None	None	Negative
18	AJM	66	246	380	10.6	3.5	0.8	200	6.4	4.7/1.7	Neg	—	1Y	Obesity. Arterio-sclerotic heart disease Traumatic ulcer foot	None	None	None	Negative
19	DOJ	40	294	450	11.4	2.9	1.4	240	7.8	4.6/3.2	Neg	Neg	3W	Obesity. Phlebitis. Arteriosclerotic heart disease.	None	None	Mod. ethanolism	Positive
20	KCM	32	370	1000	10.4	3.1	0.3	144	6.5	4.3/2.3	—	Neg	9Y	Obesity.	Proteinuria	Lente	Mod. ethanolism	Negative

with a history of chronic alcoholism and biopsy evidence of fatty metamorphosis of the liver had been diagnosed as diabetic 6 years prior to this admission. Neuropathy in this patient was manifested by impotence, absent ankle jerks, and a mild postural hypotension. Gastrointestinal evidence of neuropathy was lacking. During the previous 5 years, the patient had experienced episodes of diarrhea with up to 15 loose stools per day. Their frequency had lessened during repeated hospitalizations for control of the diabetes. His recurrent epigastric pain had been ascribed to postbulbar duodenal ulcer which had been demonstrated by Roentgen-ray 6 years previously. Pain was not a prominent feature of his many admissions. The diagnosis of chronic pancreatitis, however, had not been entertained. Correction of the diarrhea was effected with pancreatin therapy (5.4 gm. in 3 divided doses daily). Insulin requirements increased from 15 to 38 units (Lente) on a diet which was not changed with regard to caloric intake. Coincident with the pancreatin therapy was an improvement in bowel function. Labeled triolein excretion in the 48-hour stool declined from 26% to 1% and the patient gained 15 pounds on the regimen.

CASE 4. C.J.D., a 48-year-old white man who was admitted for the purpose of receiving adrenal corticosteroid treatment for neurodermatitis, was a chronic alcoholic. The diabetic state was revealed by routine blood glucose studies while he was receiving steroid therapy. Questioning elicited a history of 3 to 4 stools per day, occasionally diarrheal in character, but not otherwise grossly abnormal. The patient denied previous episodes of pain suggestive of pancreatitis and asserted that the frequent stools were not disturbing to him. There were no symptoms referable to his hyperglycemia. The increased frequency of stools was unaffected by the corticosteroid therapy but was corrected with pancreatin therapy. Diabetic control was effected with diet and chlorpropamide (Diabinese).

CASE 5. A.N., a 47-year-old white man with a diagnosis of diabetes mellitus and obesity since 1952, was admitted for treatment of symptoms resulting from an upper respiratory infection and for hyperglycemia, in addition to an anginal syndrome secondary to arteriosclerotic heart disease. Questioning revealed a history of occasional episodes of 2 to 3 "normal" stools per day, without any history of epigastric pain or distress. In addition, there was a history of moderate ingestion of alcohol for many years. The labeled fat absorption studies were incomplete due to an early discharge from the hospital, but the patient remains a suspected case of pancreatic insufficiency with a 10% excretion of labeled triolein in the 48-hour stool.

Discussion. The differential diagnosis of steatorrhea in diabetics in the absence of pancreatic calcification includes: 1) painless chronic pancreatitis, 2) primary pancreatic atrophy, 3) primary carcinoma of the pancreas and 4) the steatorrheal diarrhea complicating diabetes mellitus with neuropathy (Gross and Comfort[5]). Primary pancreatic atrophy or a small carcinoma can only be differentiated at surgical exploration. Steatorrheal diarrhea complicating diabetes mellitus with neuropathy may be recognized by the history of long-standing severe diabetes with its complications of visceral and peripheral neuritis and by failure of steatorrhea to be decreased upon administration of pancreatic extract. The data presented here support previous information reported by others (Aktan and Klotz[1]) using similar techniques in respect to the normal character of fat absorption in uncomplicated true diabetes mellitus.

The 4 proven cases of pancreatic insufficiency confirm the reports of others (Bartholomew and Comfort[2]) that chronic pancreatitis may occur without typical pain and steatorrhea may occur without overt gastrointestinal symptomatology in the presence of grossly normal appearing stools (Cameron, Bensley and Wood[3]). The one patient studied who had a proven episode of acute pancreatitis remained free of the "typical" pain pattern for 23 years, and at the time of admission only the endocrine component of his pancreatic insufficiency was prominent. The other 4 patients with abnormal triolein absorption had only vague abdominal distress which had previously been attributed to proven peptic ulceration

or ketonemia. In only one case (T.K.) did the character of the stool resemble that associated with gross steatorrhea and this was the sole patient in whom the frequency of defecation was sufficiently distressing to be reported spontaneously. The other histories of increased frequeny of stools were elicited only by direct questioning.

Four of the 5 patients diagnosed as having pancreatic insufficiency used alcohol chronically and 3 of them used it to excess. This is compatible with the long recognized role of alcohol in the etiology of chronic pancreatitis (Myers and Keefer[7]) although Bartholomew and Comfort[2] only had one case of possible alcoholism in their 10 cases of chronic painless pancreatitis.

The fact that hepatic parenchymal disease causes steatorrhea has been reviewed by Gross *et al.*[6]. However, if this steatorrhea is due to lack of bile salts for emulsification and absorption, one would not expect the dramatic response to enzyme replacement nor discrepancy in absorption of triolein and oleic acid.

This differentiation of true diabetes mellitus from that secondary to pancreatic insufficiency is essential in the comprehensive management of the individual patient. Classification of the hyperglycemia present in these cases into primary or secondary diabetes could not be made definitely. From the practical management aspect, this is of little importance since similar therapy is indicated in both types. It is conceivable that some of these cases may represent primary diabetes mellitus with complicating pancreatitis especially those with positive family histories for diabetes mellitus, however, familial pancreatitis has been reported. This latter possibility was also raised by Sprague[8] in 7 of 24 cases with positive family histories. The loss of nutrients, as well as supplemental factors such as vitamins and minerals, in this type of malabsorption state must be either corrected or compensated. Increased absorption of fat will be evidenced clinically by increasing insulin needs despite a constant caloric intake. Laboratory confirmation of this can be gained by repeating the triolein absorption studies while the patient is receiving replacement therapy with pancreatin. None of the patients reported in this study showed evidence of the avitaminosis or electrolyte disturbances previously reported in the malabsorption state secondary to pancreatic insufficiency.

The triolein absorption test offers a direct measurement of pancreatic exocrine function which heretofore has been achieved only by technically difficult balance studies or stimulation tests. Although none of these latter studies was performed, other laboratory procedures which are usually considered indicative of pancreatic insufficiency were done and of these only the presence of hyperglycemia was compatible with the diagnosis of pancreatic endocrine and exocrine insufficiency.

Summary. Twenty patients with hyperglycemia and without prominent symptoms referable to the gastrointestinal tract were studied with I_{131} labeled fat absorption tests. Four were considered to have pancreatic insufficiency as judged by the criteria of an abnormal triolein absorption pattern, a normal oleic acid absorption pattern, diabetic type hyperglycemia, and correction of the absorptive defect for triolein coincident with oral pancreatin replacement therapy. Also a fifth suspected case had an abnormal triolein absorption pattern. Further investigation disclosed that all 5 had more than one stool per day and one had gross steatorrhea; 4 of the 5 used alcohol

chronically and 3 used it to excess. Diabetic patients with a known history of chronic alcoholic ingestion should be suspected of having pancreatic exocrine insufficiency and considered candidates for a fat absorption study regardless of the duration of the diabetic state, the presence of a family history of diabetes or the apparent lack of gastrointestinal symptoms.

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REFERENCES

1. **Aktan, H. S.**, and **Klotz, A. P.**: Ann. Int. Med., **49**, 820, 1958.
2. **Bartholomew, L. G.**, and **Comfort, M. W.**: Gastroenterology, **31**, 727, 1956.
3. **Cameron, D. G., Bensley, E. H.**, and **Wood, P.**: Ann. Int. Med., **37**, 553, 1952.
4. **de La Huerga, J., Yesinick, C.**, and **Popper, H.**: Am. J. Clin. Path., **23**, 1163, 1953.
5. **Gross, J. B.**, and **Comfort, M. W.**: Am. J. Med., **21**, 596, 1956.
6. **Gross, J. B., Comfort, M. W., Wollaeger, F. E.**, and **Power, M. H.**: Gastroenterology, **16**, 140, 1950.
7. **Myers, W. K.**, and **Keefer, C. S.**: New England J. Med., **210**, 1376, 1934.
8. **Sprague, R. G.**: Proc. Staff Meet., Mayo Clinic, **22**, 553, 1947.
9. **Tietz, N. W., Borden, T.**, and **Stepleton, J. D.**: Am. J. Clin. Path., **31**, 148, 1959.
10. **Van Loon, E. J., Likins, M. R.**, and **Segar, A. J.**: Ibid., **22**, 1134, 1952.

SUMMARIO IN INTERLINGUA

Latente Insufficientia Exocrin Pancreatic in Patientes con Hyperglycemia

Vinti patientes con hyperglycemia e sin prominente symptomas attribuibile a un disordine del vias gastro-intestinal esseva studiate per medio de tests del absorption de grassia marcate con I^{131}. Quatro esseva considerate como affligite de insufficientia pancreatic secundo le criterios de un configuration anormal del absorption de trioleina, un configuration normal del absorption de acido oleic, un typo diabetic de hyperglycemia, e le correction del defecto absorptive pro trioleina como effecto de un therapia de reimplaciamento con pancreatina oral. Etiam un quinte (suspecte) caso habeva un configuration anormal del absorption de trioleina. Un investigation additional revelava que omne le 5 habeva plus que un vacuation fecal per die, e un habeva steatorrhea grossier. Quatro del 5 esseva usatores chronic de alcohol; 3 usava alcohol a excesso. Patientes diabetic con un anamnese cognoscite de ingestion chronic de alcohol deberea esser suspicite de haber insufficientia exocrin pancreatic. Tal patientes es a considerar como candidatos pro un studio del absorption de grassia, sin reguardo al duration del stato diabetic, al presentia de antecedentes familial de diabete, o le apparente manco de symptomas gastrointestinal.

A DOUBLE-BLIND STUDY OF DIETHYLPROPION IN OBESITY

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The importance of obesity in clinical medicine is known but too well. The accompanying hazards of this abnormal state and the unfavorable effect on longevity have been documented in the past (Goldner[3,4]). Only recently has an extensive study (Metropolitan Life[9], Society of Actuaries[15]) reaffirmed these conclusions. Since the anorexigenic agents play an important part in the treatment of obesity, particularly in the first 2 months (Gelvin and McGavack[2]), any new drug or new combination of older agents should be carefully assessed.

The following is a report of a double-blind study employing a placebo and diethylpropion. The importance of the blind technique for a study with a single objective measurement such as body weight, may not be readily apparent. The observation of side effects, however, makes the double-blind technique mandatory (Wolf[17], Wolf and Pinsky[18]). This represents the second such reported study with diethylpropion, the first in English literature.

Material and Methods. The patients herein described were referred mostly from other institutions. In a few instances, there were direct patient referrals to the Obesity Clinic. The degree of obesity was determined from the norms provided by the recent study of the Society of Actuaries[15].

The subjects of the current clinical investigation were members of a low socio-economic group many receiving some form of public assistance. The usual reason for the patient's obesity was a liberal ingestion of food, particularly of high carbohydrate variety. Abnormal body weight was either life-long or followed the first pregnancy.

At the first clinic visit, a complete medical history was obtained and a complete physical examination was performed. Each patient, regardless of any coexisting medical complications, such as hypertension or diabetes, was placed on a 1000 calorie diet consisting of 115 gm. of carbohydrates, 35 gm. of fat, and 59 gm. of protein and either the placebo or the diethylpropion (25 mg.).* No special instructions were given as to fluid intake. The diet was explained fully to the patient by the dietitian or nurse at the outset and at intervals during the study. The medications, identical in appearance, were dispensed in identical boxes containing a 2-week supply, to be taken one hour before meals three times daily. Patients returned for the second visit after one or 2 weeks, and then every 2 or 3 weeks subsequently. If an appointment could not be made at 2 weeks, additional medication was supplied to carry the patient until the next visit to the clinic. Upon each revisit, a history of exercise tolerance was obtained, the blood pressure and pulse were determined, the presence or absence of ankle edema was noted and the patient was questioned for side effects. At the completion of 8 weeks of therapy, or the nearest clinic visit thereafter, in addition to the revisit procedure, a second physical examination was performed. Weights were obtained by the same nurse on the same standard platform scale at the same time of day. In all cases, the patients were told that the drug was to curb their appetite, and in no instance were they aware of the existence of a research project.

The patients in the groups treated with the placebo and the active drug respectively,

*Kindly supplied as Tepanil and as an identical placebo tablet by The National Drug Company. Diethylpropion is also available as Tenuate (Wm. S. Merrell Co.).

were quite comparable in most aspects (Table 1). The group of patients on active medication averaged some 12% more overweight than those on the placebo. In brief, there were 37 patients in the study; 29 received the placebo and 28 the diethylpropion. Two courses of treatment were given to 2 patients in the active drug group. Thus, there were 29 trials with the placebo and 30 with the diethylpropion. Nineteen of the 37 patients received only one of the medications and 18 received both drugs sequentially in a cross-over experiment (Table 2).

Differences in the vital signs (blood pressure and pulse) between the two groups both at the beginning and end of therapy and also within each group at the beginning and end of therapy are minor and insignificant. These physiological data are detailed in Table 4.

Side effects were defined as untoward or abnormal symptoms appearing during the course of treatment and not present at the outset. Table 5 reveals no essential difference in the incidence of side effects between the placebo and the diethylpropion groups (14 vs. 18%, $X^2_{(1)} = 0.01$, $p<0.95$). All the side effects were transient, responding to simple pharmacological therapy or to reduction in the dosage to 2 tablets daily. Of interest is the occurrence of two instances of constipation in one patient in the placebo group and none in the group receiving

TABLE 1.—CLINICAL CHARACTERISTICS

	Placebo	*Diethylpropion*
Males:Females	1:28	0:28
White:Non-White	8:21	8:20
Range of Age	24–64	24–63
Average Age	46.2	43
Range of Weight	122.2–265.5	127.5–281
Average Weight	178.1	190.4
Range of Ideal Weight	120–151*	120–151**
Average Ideal Weight	138.7*	135.5**
Average Excess Weight	39.4*	55.1**
Range of % Overweight	2–116*	15–123**
Average of % Overweight	28.4*	40.7**
	*of 28 cases	**of 27 cases

TABLE 2.—TIME OF TREATMENT WITH DIETHYLPROPION AND WITH PLACEBO

		Avg. No. of Weeks on	
	Patients	*First Drug*	*Second Drug*
Received only diethylpropion	9	4.3	
Received only placebo	10	6.5	
Received first diethylpropion then placebo	12	9.8	7.9
Received first placebo then diethylpropion	6	8.3	9.2
	37		

Results in Entire Series. The 29 patients receiving the placebo thrice daily over an average period of 7.4 weeks lost a mean of 1.5 pounds at a rate of 0.2 pound per week. By contrast, the 28 patients receiving the diethylproprion over an average interval of 7.7 weeks lost a mean of 8.5 pounds at a rate of 1.1 pounds per week. The difference in rates of weight loss of 0.9 pound per week is statistically significant at the 0.1% level ($t_{57} = 3.5$, $p<0.001$). These results are detailed in Table 3.

the diethylpropion. The purpuric rash which developed in one patient while taking diethylpropion disappeared by the following visit without any change in medication.

Vital capacity measurements were made in 3 patients on placebo and 4 on diethylpropion. In each case there was a small, insignificant improvement during the 8-week course of medication.

Results in Subgroups. Of the 37 patients in the entire series, 19 received only one of the study preparations. Ten cases were treated with the placebo over an average period of 6.5 weeks and lost a mean of 4.6 pounds at a rate of 0.7 pound per week. By contrast,

TABLE 3.—RESULTS IN ENTIRE SERIES

	Placebo	*Diethylpropion*
Number of Patients	29	28
Number of Trials	29	30
Range of Study Time (Wks.)	2–17	2–18
Average Study Time (Wks.)	7.4	7.7
Range of Weekly Wt. loss	+1.1–2.0	+1.1–4.5
Average Weekly Wt. Loss	0.2*	1.1*
Range of Total Wt. Loss	+9–17	+5.5–34.2
Average Total Wt. Loss	1.5	8.5

*$t_{(57)} = 3.5$, $p<0.001$

TABLE 4.—PHYSIOLOGICAL DATA IN ENTIRE SERIES

	29 Cases Placebo	*28 Cases Diethylpropion*
Pretreatment (Means)		
Systolic Blood Pressure	130	134
Diastolic Blood Pressure	83	80
Pulse	75	73
Posttreatment (Means)		
Systolic Blood Pressure	125	132
Diastolic Blood Pressure	76	80
Pulse	72	73

TABLE 5.—SIDE EFFECTS

	29 Cases Placebo		*28 Cases Diethylpropion*	
	Frequency	*Week of Occurrence*	*Frequency*	*Week of Occurrence*
Headache	1	4	2	1,2
Dizziness	2	4,8	1	8
Nervousness	1	1	1	2
Insomnia			1	2
Urinary Frequency			1	6
Constipation	2	2,4		
Transitory Purpuric Rash			1	1
Fatigue	1	8		
Total Side Effects	7		7	
Total Patients	4 (13.8%)*		5 (18%)*	

*$X^2_{(1)} = 0.01$, $p<0.95$

9 patients receiving the diethylpropion over an average interval of 4.3 weeks lost a mean of 7.7 pounds at a rate of 1.8 pounds per week. The difference in rates of weight loss of 1.1 pound per week is statistically significant at the 5% level ($t_{(18)} = 2.2$, $p<0.05$). These results are detailed in Table 6.

TABLE 6.—RESULTS IN PATIENTS RECEIVING ONE PREPARATION

	Placebo	*Diethylpropion*
Number of Patients	10	9
Number of Trials	10	10
Range of Study Time (Wks.)	2–8	2–8
Average Study Time (Wks.)	6.5	4.3
Range of Weekly Wt. loss	+0.4–1.4	0.2–4.5
Average Weekly Wt. Loss	0.7°	1.8°
Range of Total Wt. Loss	+3.2–17	1.5–15
Average Total Wt. Loss	4.6	7.7

°$t_{(18)} = 2.2$, $p<0.05$

Eighteen of the 37 patients in the entire series received both drugs, 12 crossing over from diethylpropion to placebo and 6 changing over from placebo to diethylpropion. The subgroup of 12, while receiving the diethylpropion, was studied an average of 9.8 weeks and lost a mean of 8.8 pounds at a rate of 0.9 pound per week. While being treated with the placebo, they were observed an average of 7.9 weeks and gained a mean of 0.8 pound at a rate of 0.1 pound per week. This difference is statistically significant at the 0.1% level ($t_{11} = 8.3$, $p<0.001$).

The subgroup of 6 patients, started first on placebo, was studied an average of 8.3 weeks on placebo and lost a mean of 1.7 pounds at a rate of 0.2 pound per week. While being treated with the diethylpropion, they were observed an average of 9.2 weeks and lost a mean of 5.5 pounds at a rate of 0.6 pound per week. This difference is not statistically significant ($t_{(5)} = 1.5$, $p<0.5$).

Observations on vital signs and side effects have already been presented above in connection with the results in the entire series.

COMMENTS. The statistically significant difference between the weight loss observed under the diethylpropion and that under the placebo is worthy of explanation. Dextro-amphetamine, in use for more than a decade, represents the oldest commonly used anorexigenic agent. This drug results in a diminished food intake via a direct influence on the brain (Goodman and Gilman[5]), but appears to lose its effectiveness with prolonged administration, particularly after the eighth week. Gelvin and McGavack[2] demonstrated this most strikingly in a group of patients without dietary restriction. Reasons for the decline in the rate of weight loss include tolerance to the drug and insufficient decrease in the caloric intake (Gelvin and McGavack[2]). Because of these limitations and the equally important side effects resulting from central nervous system stimulation, the search for more satisfactory anorexigenic agents, such as diethylpropion, continues.

This drug, developed in Germany (Huels[6], Illig and Illig[7], Schuppius[14]) has only recently been reported from American centers (Decina and Tanyol[1], Martin[8], Ravetz[11], Spielman[16]). Most of the available literature on diethylpropion appears in a recent symposium (Michigan Academy of General Prac-

tice[10]) on overweight and underweight. Diethylpropion (1-phenyl-2-diethyl-amino-propanone-1-hydrochloride) like amphetamine contains the beta-phenylethylamino basic structure (Martin[8]). Experimentally, it was shown to have minimal undesirable side effects arising from central nervous system stimulation as compared to dextro-amphetamine (Martin[8]). Likewise, toxicity studies have revealed a great margin of safety in its use (Martin[8]).

In single drug studies, that is, where patients received either diethylpropion or a placebo weekly, rates of weight loss of 1.3 (Decina and Tanyol[1], Spielman[16]) and 1.7 pounds (Ravetz[11]) under the active drug and 0.4 (Spielman[16]) and 0.5 (Decina and Tanyol[1]) pound under the placebo have been observed. In our experience reported above, these figures were comparable, 1.8 for the diethylpropion and 0.7 for the placebo.

Two reports (Huels[6], Illig and Illig[7]) of cross-over studies with diethylpropion and a placebo are available in the literature. They are not comparable to ours, in that one (Huels[6]) involved 10 patients with various organic diseases in addition to obesity, who were treated sequentially in the hospital and outpatient department. The periods of observation were both shorter (8 to 12 days) and longer (3 months) than in ours, but weight loss was satisfactory. The other reported cross-over study is that of Illig and Illig[7] who observed 48 diabetics over 4-week periods; their figures are 50% significant weight loss (2.2 pounds per 4 weeks) under diethylpropion and 28% under the placebo. Our 12 patients who crossed over from the active drug to the placebo lost a mean of 0.9 pound per week and gained a mean of 0.1 pound per week respectively. Employing the criterion of Illig and Illig[7], 50% of our patients lost a significant amount of weight under diethylpropion and 0% after crossing over to the placebo.

In our opposite cross-over study involving 6 patients changing from placebo to active drug, no significant difference was observed (0.2 vs. 0.6 pound per week). However, this discrepancy may be resolved as follows. First, the number of patients (6) was relatively small as compared to the opposite cross-over study (12). Second, the 3 patients who gained weight while under the placebo, actually lost in 2 cases or gained at a lower rate in one case when taking the diethylpropion. Third, initial discouragement with a placebo over an 8-week period might easily carry over when the active drug is taken. Finally, the first weeks of any reducing regimen are more productive of weight loss than later weeks (Gelvin and McGavack[2]).

Lack of adverse effect of diethylpropion on the blood pressure and pulse as noted in our patients has also been observed by others (Decina and Tanyol[1], Illig and Illig[7], Ravetz[11]). The same is true of side effects in both our experience and that just quoted (Decina and Tanyol[1], Illig and Illig[7], Ravetz[11]). This study illustrates again the importance of the double-blind control method of drug evaluation in the consideration of untoward symptoms. Thus, in previous work in this clinic (Rosenberg[12,13]), a similar incidence of side effects had been observed in obese patients under treatment with an active drug and a placebo. Striking in this study was the occurrence of the 2 instances of constipation alone in the placebo group and such central nervous system effects as headache, dizziness, and nervousness in both groups. This is another demonstration of the powerful placebo effect of any medication, regardless of its potency or pharmacological action and

the fact that placebos may work both positively (Wolf[17]) and negatively (Wolf and Pinsky[18]). The importance of the double-blind technique of drug investigation in humans becomes obvious.

Summary. A double-blind study of diethylpropion in 37 patients in an Obesity Clinic has been reported. The mean weekly weight loss was 1.1 pounds in the 28 patients receiving the active drug and 0.2 pound in the 29 patients receiving the placebo. Among the 19 patients being treated with only one drug, the mean weekly weight loss was 1.8 pounds in the diethylpropion group and 0.7 pound in the placebo group.

In the entire series, transient minor side effects occurred in 18% of the patients receiving diethylpropion and in 14% of those receiving the placebo. No major untoward effects occurred in either group.

The value of the double-blind technique in drug evaluation has again been reaffirmed.

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REFERENCES

1. **Decina, L.**, and **Tanyol, H.**: New York J. Med., **60**, 2702, 1960.
2. **Gelvin, E. P.**, and **McGavack, T. H.**: Obesity, Its Cause, Classification and Cure. New York: Paul B. Hoeber, Inc., 1957.
3. **Goldner, M. G.**: Med. Clin. N. America, **39**, 1, 1955.
4. Idem: New York J. Med., **56**, 2063, 1956.
5. **Goodman, L. S.**, and **Gilman, A.**: The Pharmacological Basis of Therapeutics. New York: Macmillan Co., 1955.
6. **Huels, H. G.**: Sixth Spring Symposium on Overweight and Underweight, Detroit, Michigan Academy of General Practice, p. 57, 1959.
7. **Illig, A.**, and **Illig, H.**: Medizinische, **22**, 1077, 1959.
8. **Martin, G. J.**: Sixth Spring Symposium on Overweight and Underweight, Detroit, Michigan Academy of General Practice, p. 5, 1959.
9. Metropolitan Life Insurance Company: New Weight Standards for Men and Women, Statistical Bulletin, **40**, 1, 1959.
10. Michigan Academy of General Practice: Sixth Spring Symposium on Overweight and Underweight, Detroit, March 4, 1959.
11. **Ravetz, E.**: Ibid., p. 99.
12. **Rosenberg, B. A.**: Curr. Ther. Res., **3**, 45, 1961.
13. Idem: Unpublished observations.
14. **Schuppius, A.**: Aerztl. Praxis, **10**, 1242, 1958.
15. Society of Actuaries: Build and Blood Pressure Study, Vol. 1, Chicago, 1959.
16. **Spielman, A. D.**: Sixth Symposium on Overweight and Underweight, Detroit, Michigan Academy of General Practice, p. 39, 1959.
17. **Wolf, S.**: J. Clin. Invest., **29**, 100, 1950.
18. **Wolf, S.**, and **Pinsky, R. H.**: J. Am. Med. Assn., **155**, 339, 1954.

(Interlingua on page 172)

THE RESPONSE OF HYPOPROTHROMBINEMIA IN CIRRHOSIS TO VITAMIN K_1 AND K_3*

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Numerous naphthoquinone compounds exhibit vitamin K activity (Brinkhaus[3]). They differ from each other in potency, duration of action, and solubility in water and lipid. Until recently only the water soluble vitamin K (K_3; 2-methyl-1,4-naphthoquinone; Synkayvite; Hykinone) was available for parenteral administration in man. The value of this form of the vitamin in the treatment of cirrhosis (Unger, Weiner and Shapiro[8]) and in counteracting coumarin-induced hypoprothrombinemia (Shapiro, Weiner and Simson[6]) is limited.

Recently developed formulations of water miscible vitamin K_1 (2-methyl-3 phytyl-naphthoquinone; Mephyton; Konakion) permitting intravenous injection, exert a clearly more potent and longer lasting influence against coumarin-induced hypoprothrombinemia (Barker[2]) as compared to K_3. This study was designed to determine whether one of these new formulations of vitamin K_1 (Aqua Mephyton, Merck Sharp and Dohme) is also more effective in correcting the hypoprothrombinemia associated with hepatic cirrhosis.

Liver disease, coumarin anticoagulants, and vitamin K deficiency may influence several of the stable components of the prothrombin complex. In this study, prothrombin activity was estimated both by the usual one stage method using rabbit lung-brain thromboplastin (Simplastin, Warner-Chilcott Labs.) (Shapiro *et al.*[5]), which is sensitive primarily to prothrombin and proconvertin activity, and the Russell venom thromboplastin (Stypven, Burroughs Wellcome), which reflects more closely changes in the Stuart factor (Bachmann, Duckert and Koller[1]). Both whole and 12.5% saline diluted plasma were studied in order to increase sensitivity and detect possible heparinoid activity (Shapiro and Weiner[4]). Recalcification time (Weiner and Jiminez[10]) and antithrombin activity (Weiner[9]) were also estimated.

Nine hospitalized cirrhotic patients were studied. Eight were moderately to severely cirrhotic, and in one the diagnosis of cirrhosis was questionable.

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TABLE 1.—EFFECT OF VITAMIN K_1 AND K_3 ON PROTHROMBIN TIME (SECONDS) IN 8 CIRRHOTIC SUBJECTS

Undiluted Plasma						*12.5% Saline Diluted Plasma*					
*Rabbit Thromboplastin (13.5)**			*Viper Venom (20.5)**			*Rabbit Thromboplastin (31.5)**			*Viper Venom (46.0)**		
Control	*After K_3*	*1 Month later*	*Control*	*After K_3*	*1 Month later*	*Control*	*After K_3*	*1 Month later*	*Control*	*After K_3*	*1 Month later*
20.0	16.5	17.5	27.0	28.5	25.0	46.0	48.0	57.5	140.0	75.0	67.5
17.0	19.0	15.5	22.0	22.0	24.0	45.0	43.5	49.0	125.0	52.0	56.0
16.5	15.0	16.5	23.0	22.5	24.0	45.5	39.5	43.5	69.5	68.5	80.5
15.0	14.0	14.0	23.0	23.5	26.0	38.5	38.0	36.5	72.5	83.0	61.0
14.5	14.0	14.0	22.5	19.5	22.5	31.5	28.5	32.0	57.0	48.5	52.5
15.5	13.5	13.0	23.0	18.5	23.0	35.0	30.5	36.0	70.5	59.0	45.5
15.5	15.0	13.5	25.0	25.0	25.5	41.0	35.5	34.0	61.5	62.5	52.5
15.5	14.5	14.5	22.5	22.5	23.5	39.0	38.5	34.5	53.0	54.5	52.0
Av. 15.9	15.1	14.8	23.5	22.7	24.1	40.1	35.8	40.3	81.1	62.8	58.4

After K_1	*1 Month later*
25.5	29.5
25.5	16.5
23.5	21.5
22.5	21.5
21.5	25.5
18.0	20.5
23.5	23.0
20.5	24.5
22.5	21.5
39.5	40.3	34.3	37.1	50.5	61.0

*Average values in normal human subjects

One died during the period of observation. During a control period of one week, 3 blood specimens were obtained and studied, following which 75 mg. of vitamin K_3 (Synkayvite) were administered intramuscularly every other day for 5 doses, and coagulation studies were performed 24 hours after the third, fourth, and fifth dose. No further medication was given for 25 to 30 days, after which new control specimens were studied, followed by estimated with Simplastin) was complete. However, a month after K_1 therapy, only a questionable degree of relapse of prothrombin deficiency was noted. Two of the subjects were initially found to have a rather marked deficiency by the viper venom method. This deficiency was largely corrected by the initial doses of vitamin K_3, and remained in the corrected range throughout the rest of the experiment. Evaluation of the further reduction in

TABLE 2.—EFFECT OF REPEATED DOSES OF VITAMIN K_1 ON THE PROTHROMBIN TIME OF 12.5 % SALINE DILUTED PLASMA IN 8 SUBJECTS WITH CIRRHOSIS

Rabbit Thromboplastin			*Viper Venom*		
Control	*After Third Dose*	*After Sixth Dose*	*Control*	*After Third Dose*	*After Sixth Dose*
57.5	44.0	37.0	67.5	66.0	58.0
49.0	31.5	37.5	56.0	43.5	70.5
43.5	37.0	40.0	80.5	44.5	49.5
36.5	31.0	33.5	61.0	40.5	55.0
32.0	30.0	31.5	52.5	48.0	45.0
36.0	25.5	26.5	45.5	39.5	53.5
34.0	35.5	34.0	52.5	41.5	55.5
34.5	35.0	33.5	52.0	42.0	53.5
Avg. 40.3	33.7	34.1	58.4	45.7	55.0

10 mg. of Aqua Mephyton intravenously every other day for 6 doses with further blood studies after the third, fourth, and sixth dose.

Table 1 compares the average prothrombin time of each subject in the control period with the average of the values obtained after dosage. The average control prothrombin activity as estimated with undiluted plasma was only questionably less than normal, and the changes induced by vitamin K are not statistically significant. In contrast, the more sensitive 12.5% saline diluted plasma showed a significant prolongation of prothrombin time above normal in the control period, with significant fall toward normal in response to both vitamins K_3 and K_1. One month after K_3 treatment, relapse from the improved state (as diluted plasma venom thromboplastin time by vitamin K_1 (from an average of 58.4 seconds to 50.5 seconds) is complicated by the fact that excessive dosage may paradoxically prolong prothrombin time (see below and Table 2).

It has previously been reported (Steigmann *et al.*[7], Unger, Weiner and Shapiro[8]) that repeated large doses of vitamin K may cause an aggravation of the prothrombin deficiency in liver disease. In this study, the phenomenon was observed in response to both K_1 and K_3 in some subjects. Only the diluted plasma snake venom thromboplastin results in the K_1 portion of this study showed, for the group as a whole, a significant increase in the prothrombin time by 3 additional doses of K_1 after a shortening had been achieved by 3 initial doses (Table 2).

These data confirm that the prothrombin complex deficiency in some cirrhotic subjects may be partially corrected by either vitamin K_1 or K_3. This effect is more readily detected with diluted plasma and apparently persists longer after K_1 than K_3. The correction achieved by a series of 3 injections of vitamin K_1 is partially reversed in some subjects by 3 further injections. This is reminiscent of previous observations concerning response to repeated high doses of vitamin K_3. No conclusions could be drawn from the data concerning recalcification time and antithrombin activity.

Summary. Prothrombin complex deficiency in some cirrhotic subjects may be partially corrected by either vitamin K_1 or K_3. This effect is more readily detected with diluted plasma and apparently persists longer after K_1 than K_3. The correction achieved by a series of three injections of vitamin K_1 is partially reversed in some subjects by three further injections. This is reminiscent of previous observations concerning response to repeated high doses of vitamin K_3.

REFERENCES

1. **Bachmann, F., Duckert, F.,** and **Koller, F.:** Thromb. diath. haem., **2,** 24, 1958.
2. **Barker, N. W.:** Anticoagulant Therapy. Proc. First Internat. Cong. Thrombosis and Embolism. Basel: Benno Schwabe and Co., p. 683, 1955.
3. **Brinkhaus, K. M.:** Medicine, **19,** 329, 1940.
4. **Shapiro, S.,** and **Weiner, M.:** Internat. J. Hem., **3,** 16, 1950.
5. **Shapiro, S., Weiner, M., Luddecke, H., Kroc, R.,** and **White, E.:** Am. Heart J., **40,** 766, 1950.
6. **Shapiro, S., Weiner, M.,** and **Simson, G.:** New England J. Med., **243,** 775, 1950.
7. **Steigmann, F., Schrifter, H., Yiotsas, Z. D.,** and **Pamukcu, F.:** Am. J. Gastroenterol., **31,** 369, 1951.
8. **Unger, P., Weiner, M.,** and **Shapiro, S.:** Am. J. Clin. Path., **18,** 835, 1948.
9. **Weiner, M.:** Clin. Chem., **4,** 271, 1958.
10. **Weiner, M.,** and **Jiminez, M.:** Am. J. Clin. Path., **26,** 243, 1956.

SUMMARIO IN INTERLINGUA

Le Responsa de Hypoprothrombinemia, in Casos de Cirrhosis, a Vitamina K_1 e K_3

Carentia de complexo prothrombinic pote, in certe subjectos cirrhotic, esser corrigite partialmente per vitamina K_1 o per vitamina K_3. Iste effecto es plus prestemente detegite con plasma diluite. Apparentemente illo persiste plus longo post vitamina K_1 que post vitamina K_3. Le correction effectuate per un serie de tres injectiones de vitamina K_1 es partialmente revertite in certe subjectos per tres injectiones additional. Isto reappella al mente previe observationes con respecto al responsa a repetite alte doses de vitamina K_3.

DEGRANULATION OF THE BASOPHIL IN MAN INDUCED BY ALIMENTARY LIPEMIA*†

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In the last few years there has been a tremendous surge of publications in the field of atherosclerosis. Articles, monographs and indeed new journals form a virtual literary cascade, with a central stream of lipid chemistry and metabolism. In this regard, Fritz has given us a good modern compilation of reference sources (Fritz[12]). At present no area appears to be receiving more attention here than that of fat transport. Two recent splendid reviews (Robinson and French[28], Olson and Vester[26]) summarize our knowledge, and it is to this area that we would like to direct attention. After a fatty meal, individuals normally show a cloudy serum as optical evidence of the blood transport of absorbed fat in the form of relatively insoluble lipoproteins (chylomicrons). Over a period of hours, such serum "clears" and the chylomicron count drops to low levels. However, many studies have established that the atherosclerotic group has a uniquely prolonged period of such postalimentary lactescence, thus pointing up a possible defect in fat clearing in these patients (Bouchier and Bronte-Stewart[6]).

In 1943, Hahn[14] made the serendipitous but significant discovery that the anticoagulant heparin strangely entered into this picture of fat transport. He found that the injection of heparin led to rapid clearing of milky lipemic serum. Within the next dozen years, it was learned that the heparin did not operate directly or *in vitro*, but actually released a new factor, lipemia-clearing factor, which could clear lipemic plasma as well as pure triglyceride emulsions either *in vitro* or *in vivo*. Largely due to the work of Korn[23] it is known that the lipemia clearing factor is an enzyme, lipoprotein lipase, which splits the triglyceride moiety of chylomicrons and low density lipoproteins to fatty acids and glycerol. Such fatty acids form a soluble complex with serum albumin and it is this change in fat transport from the light scattering chylomicrons to the invisible albumin complex which accounts for the clearing phenomenon. Without heparin, however, lipoprotein lipase is found

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only in negligible amounts in plasma (Kern, Steinmann and Sanders[22]).

Although administered heparin can induce such a sequence of events, the question remains: Does lipemia of itself mobilize endogenous heparin and so initiate an automatic clearing reaction? This question could best be answered by determining heparin levels before and after a fatty meal. Unfortunately, however, direct chemical estimation of heparin is not possible and its estimation by coagulation studies is unsatisfactory after a fatty meal due to participation of the phospholipids in the complex clotting process (O'Brien[25]). Thus, it is necessary at present to attempt an indirect approach. The mast cell would seem to hold an answer since it has long been recognized as the cellular reservoir of heparin (Jorpes[18]). Jennings, Florey and Robinson[17] have made intensive studies of this cell in the rat, but so far have failed to demonstrate its participation in lipemic fat transport. Watson[33] and ourselves[29] in a series of experiments have likewise been unable to adduce morphologic or physiologic evidence that the rat mast cell heparin is mobilized as a result of lipemia.

Turning from this defeat, we realized that there is another cellular source of heparin, namely, the basophilic granulocyte. Here is a congener of the mast cell, one showing the same metachromatic staining with aniline dyes which is indicative of the presence of heparin. Indeed in 1953, Martin and Roka[24] adduced more direct evidence that the basophil in man contains a heparin-like substance. They isolated a heparin-like anticoagulant from the acetone extract of the leukocytes of a case of basophil leukemia. Behrens and Taubert[3] had earlier experimental data indicating the basophil in the horse contains heparin. Such chemical parallelism in these two cell lines is not altogether unexpected since both the mast cell and the basophil are unquestioned reservoirs of histamine. In any event, the blood-borne basophil is a likely source for heparin. Thus we have addressed ourselves to the problem by studying the effect of test meals on the cytology of the circulating basophil in man. It seemed likely that, if the clearing reaction is a basic or intrinsic physiologic regulatory mechanism, lipemia might induce morphologic changes in this cell as evidence of heparin mobilization into the blood stream.

Methods. Normal healthy young adult males, Negro and white, served as experimental subjects. All were inmates of a penal institution and under reasonably close dietary control. Test feedings were given at intervals of one week to allow for re-establishment of any equilibrium disturbed at the time of testing. On the day of the experiment only the foods detailed below were given. The men were allowed to drink water *ad lib*, but not permitted to exercise. In each instance test findings were preceded by an 18-hour overnight fast.

Since the basophil is both fragile and rare, special techniques had to be developed for its handling, fixation and concentration. Reference may be made elsewhere for the details (Shelley and Juhlin[31]), but in principle venous blood is drawn into a siliconized needle and syringe, forcibly ejected into ice bath chilled organic solvent fixative (acetic acid, ethyl alcohol, chloroform). This destroys all of the erythrocytes, leaving only the leukocytes. After a minimum 18-hour storage period in a deep freeze cabinet, half of the supernatant fixative is removed, the precipitated cells redispersed, and aliquots filtered through coarse (Cella O, 20 mm.) membrane filters of cellulose* using a micro suction unit. The filter with its adherent cells is then stained in aqueous toluidine blue (0.3%), dehydrated in ethyl alcohol, cleared in xylene, and mounted on microscope slides. It is then possible to study the morphology of the basophils, which show specific dark blue, as well as metachromatic staining of the granules. Under a magnification of 645×, we routinely count and classify 40 consecutive basophils using the rather distinctive

*Schleicher and Schuell, Keene, New Hampshire.

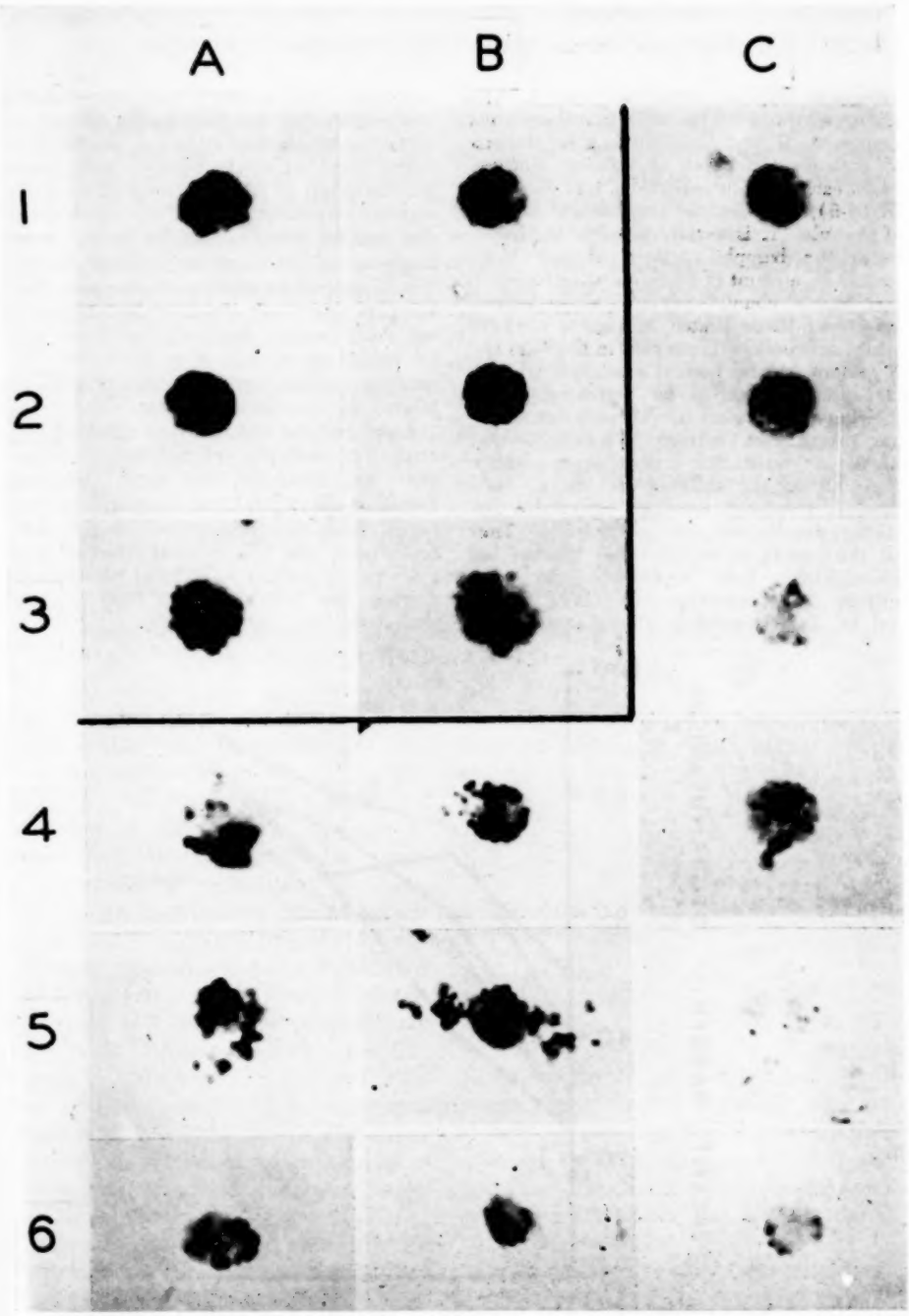

Fig. 1.—*Basophil differential* used in the analysis of morphologic changes during lipemia. Each of 40 consecutive basophils is classified into one of the above 18 types. Such a differential count permits ready visual detection of degranulation. The six cells above and to the left of the black line are considered normal. The other 12 show varying degrees of degranulation. The number of cells falling into these latter groups form the basis for the calculation of the percentage of degranulated cells. Toluidine blue stain, magnification 1750×.

differential typing shown in Fig. 1. This typing separates the basophils into three major groups, A, B, and C, depending on the size of granules and depth of staining. Furthermore, each group is subdivided into six classes (1 to 6), reflecting the number and location of granules. It is usually possible to characterize the basophil pattern of any blood sample by a count of 20 consecutive basophils and repeat counts regularly show general agreement. As indicated in Fig. 1, we have arbitrarily considered the cells in the $A_{1,2,3}$ and $B_{1,2,3}$ series to be normal examples, whereas the other cells may be presumed to be showing degranulation. This is an arbitrary separation, but it is evident that it is a satisfactory first approximation since all of the cells of the $A_{4,5,6}$, $B_{4,5,6}$, $C_{1,2,3,4,5,6}$ groupings have an appreciable loss in the number or size of granules. Thus in the data presented, the number of degranulated cells represents the total number or percentage of $A_{4,5,6}$, $B_{4,5,6}$, and $C_{1,2,3,4,5,6}$ counted in the analysis of 40 basophils. Under short term experimental analysis, we find this comparative rating technique to be satisfactory. As a second technique, more elaborate surveys with photographic prints of each cell have given results in good agreement. A third way of observing the basophil response involves recording the frequency of cell types in a scatter diagram. Visual comparison of these "before and after" basophil count patterns also permits one to see shifts toward degranulation. These may be graded on a scale of + to ++++, depending on the degree of differentiation exhibited by the count patterns.

Lipid analyses included total esterified fatty acids (Reinhold[27]), phospholipids (Zilversmit and Davis[35]) and total cholesterol (Abell *et al.*[1]). Total triglycerides were computed using the formula: triglyceride fatty acid (mEq. per L.) = total esterified fatty acids (mEq. per L.) − [0.58 × phospholipid P (mg. per 100 ml.) + 0.0053 × total cholesterol (mg. per 100 ml.)].

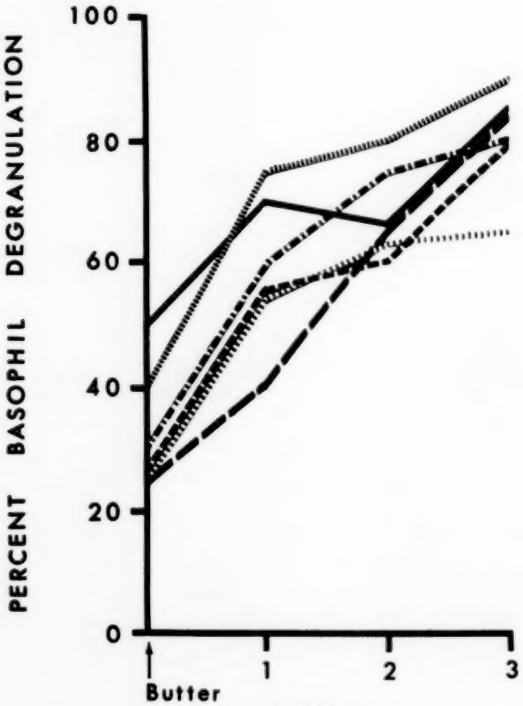

Fig. 2.—Basophil degranulation as observed in 6 subjects following the ingestion of butter in large quantity (4 gm. per kg.). By the end of 3 hours most of the cells were significantly depleted of granules.

Results. (a) BASIC DEGRANULATION RESPONSE TO SINGLE TEST FEEDING OF BUTTER. Six men were fed a single meal of butter in the amount of 4 gm. per kilogram of body weight. The only other food given was white bread in an amount of from 8 to 10 slices, depending on the wishes of the subjects. The butter and bread were consumed in 40 minutes on the average. Figure 2 shows the total percentage of degranulated cells (Type A_4, A_5, A_6, B_4, B_5, B_6, C_1, C_2, C_3, C_4, C_5, C_6) at the beginning of the experiment and at hourly periods thereafter. The data are presented for each man.

TABLE 1.—EFFECT OF SINGLE MEAL OF VARIOUS FOODS ON THE BASOPHIL

Meal 4 gm./kg.	*Percentage of Degranulated Basophils**			
	Fasting 18 hours	*Time after the start of eating*		
		1 hour	*2 hours*	*3 hours*
Butter	31.7 ± 2.6	59.0 ± 5.0	68.5 ± 3.0	80.5 ± 2.
Sugar	37.5 ± 6.4	32.5 ± 6.0	37.5 ± 6.4	49.0 ± 6.0
Chicken	54.0 ± 2.4	61.5 ± 6.0	58.3 ± 5.7	56.0 ± 2.7

* The figures given here and in the following tables indicate arithmetic mean and standard error thereof for a team of six men.

(b) COMPARATIVE DEGRANULATION RESPONSE TO FAT, CARBOHYDRATE AND PROTEIN TEST FEEDING. A second team of 6 men was fed a single meal of butter, sugar and chicken breast respectively at intervals of one week. The quantity in each instance was 4 gm. per kilogram of body weight. The butter was served with bread as before, the sugar dissolved in water with addition of a flavoring agent, and the chicken breast served cold after roasting. Table 1 summarizes the mean data obtained on the 6 men. It can be seen that butter is uniquely associated with postprandial basophil degranulation.

(c) COMPARISON OF ANIMAL AND VEGETABLE FAT DEGRANULATION RESPONSE. A team of 8 men was test fed single meals of butter, margarine and bread respectively at intervals of one week. The butter, as well as the margarine, was served with bread and the bread (8 to 10 slices) alone served as a control feeding. The butter used was a regular creamery butter. The margarine was a standard commercial product made from corn oil (Fleischmann's). The Fleischmann Laboratories list the following lipid composition for their product in comparison with butter:

Fatty Acids	*Lipid Content in 100 gm. Fat*	
	Corn Oil Margarine	*Butter*
Mono-unsaturated:		
Oleic Acid	48	33
Polyunsaturated:		
Linoleic Acid	16	3
Saturated	16	55

This margarine also contained small amounts of vitamin A and D, lecithin, skim milk, carotene and diacetyl. Table 2 shows that the margarine and butter were equally effective in inducing basophil degranulation in these same 8 subjects.

(d) DEGRANULATION RESPONSE FOLLOWING SMALLER AMOUNT OF FAT. A team of 5 men was given a test feeding of butter in the quantity of 1 gm. per kg., and Table 3 summarizes the results. The basophil degranulation response was present, although not as

striking as in Table 1 and apparently was slower in appearing.

(e) SIX-HOUR DEGRANULATION TIME CURVE WITH COMPARISON OF *in Vivo* AND *in Vitro* OBSERVATIONS. Using 11 men, a new series of observations was made on subjects receiving a high fat meal. Table 4 summarizes the data. It was found that degranulation persisted at 6 hours, even appearing more prominently. Although the subjects received butter and margarine on a random alternate basis, no difference could be seen between the behavior of the basophil.

Inasmuch as the time a basophil remains in the blood stream may be short (Bessis[4]), a study was made to see if the degranulation reaction would be more marked in lipemic blood standing for 15 minutes at room temperature. Thus aliquots of the same heparinized blood sample (1.5 units per ml.) were allowed to stand in polystyrene tubes at room temperature 26° C. The control specimens were fixed instantly. Table 4 reveals no change in the degree of response as a result of this *in vitro* period.

(f) QUALITATIVE CORRELATION OF BASOPHIL DEGRANULATION WITH SERUM LIPID LEVELS. A team of 20 healthy subjects was fed a single meal of fat (4 gm. per kg.). The basophils were

TABLE 2.—COMPARISON OF BASOPHIL DEGRANULATION FOLLOWING BUTTER AND CORN OIL MARGARINE

	Mean Percentage of Degranulated Basophils (8 Men)	
Meal	*Fasting 18 hours*	*3 hours Postprandial*
Butter 4 gm./kg.	40.6 ± 5.9	58.9 ± 3.5
Margarine 4 gm./kg.	36.9 ± 4.2	60.0 ± 2.8
Bread 10 slice	43.1 ± 5.9	33.1 ± 3.1

TABLE 3.—PERCENTAGE BASOPHILS SHOWING DEGRANULATION AFTER FEEDING OF BUTTER IN LESSER AMOUNT (5 MEN)

Meal	*Fasting Level*	*1 hour*	*2 hours*	*3 hour*
Butter 1 gm./kg.	47.0 ± 4.6	49.0 ± 5.8	59.0 ± 5.8	68.0 ± 5.5

TABLE 4.—DEGRANULATION TIME CURVE AND IN VIVO VERSUS IN VITRO STUDIES

	Percentage Degranulated Basophils (11 Men)		
		Postprandial Butter or Margarine (4 gm./kg.)	
	Fasting	*3 hours*	*6 hours*
In Vivo: Immediate Fixation	46.8 ± 4.6	62.3 ± 3.5	71.4 ± 3.8
In Vitro: Heparinized Samples 15′ Room Temperature	46.8 ± 5.9	65.0 ± 4.1	not done

studied before feeding and 3 hours after, and the degree of degranulation change visually noted on a + to ++++ scale. At the 3-hour period, blood samples were analyzed for total esterified fatty acids, phospholipids and total cholesterol. Table 5 details the results. The degree of individual variation not seen in the previous "means" becomes evident. Some subjects do not show any degranulation detectable with our differential count technique. Significantly nearly all of these "non-reactors" had triglyceride levels in the normal fasting range, suggesting that, despite massive oral feeding of fat, their absorption was slow. The majority of subjects showed degranulation in association with high fat levels. Two others, however, showed no degranulation despite associated high triglyceride levels. The complete breakdown is:

no	degranulation	7 subjects
+	"	2 "
++	"	7 "
+++	"	2 "
++++	"	2 "

Correlation was not seen with the phospholipid or cholesterol levels. No difference could be seen between margarine and butter.

TABLE 5.—RELATION OF BASOPHIL DEGRANULATION AND SERUM LIPID LEVELS 3 HOURS AFTER HIGH FAT MEAL

Data arranged in order of ascending Triglyceride levels.
Even numbered subjects received butter; odd, margarine (4 gm./kg.)

Subject Number	*Degree of Basophil Degranulation*	*Triglyceride mEq./L.*	*Phospholipid P mg./100 ml.*	*Total Cholesterol mg./100 ml.*	*Lactescence*
12	0	5.3	9.5	245	3+
13	0	5.9	11.2	230	3+
6	0	6.6	7.7	157	2+
15	++	7.0	9.9	219	3+
3	0	7.2	10.9	258	4+
11	0	7.7	10.8	170	2+
8	++	7.9	8.8	153	3+
5	++	8.0	10.8	230	2+
10	++	9.3	11.2	260	3+
14	+++	9.6	9.0	194	4+
20	++++	10.6	9.0	170	4+
1	++	11.4	16.0	265	4+
7	+++	11.7	9.5	237	4+
16	++++	12.7	9.6	200	3+
19	+	13.0	7.2	265	4+
9	+	13.8	7.6	170	4+
4	++	14.1	8.1	200	4+
18	0	15.3	9.1	186	4+
2	++	17.7	12.1	202	3+
17	0	20.7	11.7	272	4+

(g) COMPARISON OF DEGRANULATION FOLLOWING DIET, CORTISONE AND HISTAMINE LIBERATORS. The same 20 men were studied in terms of their 3-hour response to bread (8 to 10 slices), a single oral dose of cortisone acetate (200 mg.) and finally the response *in vitro* of their basophils to two well known histamine liberators, phospholipase A (Lecithinase A)* and com-

*Prepared from bee venom and kindly supplied by Dr. Bertil Högberg, A.B. Leo, Hälsingborg Sweden.

pound 48/80.† In the latter experiment, heparinized blood samples were kept at room temperature in polystyrene micro-centrifuge tubes for 15 minutes. Physiological saline solution was used as a control. Final drug concentrations were 1/10.000. The data are tabulated in Table 6. An additional 4 men are shown at the foot of the table as examples of individuals with a marked basophil response to lipemia.

It can be seen that bread and cortisone were without significant effect. The basophil response to phospholipase A appears at least in these preliminary data to correlate with the response seen after a fatty meal. In addition, a large percentage of the subjects showed "melting" of the basophil granules under the influence of phospholipase A *in vitro*. Finally, compound 48/80 has a classic degranulating effect in over half of the subjects.

TABLE 6.—COMPARISON OF DEGRANULATION FOLLOWING DIET, CORTISONE AND HISTAMINE LIBERATORS

Subjects listed according to degree of degranulation noted following fat meal
The first twenty are the same men as in Table 5.

	Basophil Degranulation (Scored on 0 to ++++ Scale)				
	3 Hour Effect			*15 Minutes in Vitro Effect*	
Subject Number	*Fat (4 gm./kg.)*	*Bread (10 slices)*	*Cortisone (200 mg.)*	*Phospholipase A 1/10,000*	*48/80 1/10,000*
11	0	0	0	0	0
3	0	0	0	0	+
6	0	0	0	0	+++
17	0	0	0	0	++
18	0	0	0	0	0
13	0	0	0	0	+
12	0	0	0	0	+++
19	+	0	0	0	0
9	+	0	0	0	0
5	++	0	0	0	+++
15	++	0	0	0	++
1	++	0	0	0	0
4	++	0	0	0	+++
10	++	+	0	0	0
8	++	0	0	0	0
2	++	0	0	++	+++
14	+++	0	0	++	0
7	+++	0	0	++	++
16	++++	0	0	+	+
20	++++	0	+	++++	++++
21	++++	0	0	+++	++
22	++++	0	0	++	+++
23	++++	0	0	++++	++++
24	++++	0	0	++++	++

Discussion. Recent reviews of the basophils (Speirs[32], Boseila[5], Fredericks and Maloney[10]) have stressed the paucity of factual knowledge concerning the function of this cell. Nevertheless, the granules of the basophil suggest a secretory process and the chemistry of

†Kindly supplied by Burroughs-Wellcome Research Laboratories, Tuckahoe, New York.

the granules brings to mind three areas of exploration. First, the basophil granules contain histamine (Graham *et al.*[13]). Secondly, these same granules apparently contain heparin, although here the evidence is not as complete (Martin and Roka[24], Behrens and Taubert[3], Speirs[32], Kelsall and Crabb[21]) as it is for the cousin cell line, the tissue mastocytes. In any event the chemical and staining parallelism between the basophils and mast cells is so remarkable as to lead us to predict that the new discovery of fibrinolysin in the mast cells (Ende and Auditore[9]) may be followed by the demonstration of fibrinolysin in the basophils. With these two and possibly three key pharmacodynamic agents present in the granule, it is easy to see the potential significance of the basophil degranulation we have observed after a fatty meal.

The roles of heparin in the fat clearing reaction and in blood coagulation are well known. Histamine may possibly exert an equally important protective function by causing vasodilation, by inducing increased reticuloendothelial pick-up of chylomicrons, and by promoting permeability of the capillaries to the smaller fat particles. Thus, heparin would function largely in the removal of fat from the blood by degradation and histamine by mechanical removal (French, Morris and Robinson[11]). Finally, to extrapolate from the mast cell, one can perceive that the basophil may be a circulating source of plasminogen and thus of considerable importance in the problem of intravascular clot or fibrinolysis.

We visualize that the basophil, in view of its role in fat transport, may be a critical determinant in the pathogenesis of atherosclerosis. Here is a cell deserving of much more attention in the cardiovascular field. To date, however, the basophil has remained largely in the shadow of the other leukocytes, exciting attention only when it emerged in the large numbers heralding leukemia. Most of the thousands of blood cell counts done daily fail to even bring the cell into clinical view. Our preliminary observations indicate that some patients with hyperlipemia have nonresponsive basophils in the "fat test" described above. Furthermore, their basophils show no response to phospholipase A. Studies are needed to ascertain the predictive value of the basophil response to fat in the field of atherosclerosis. Are those individuals with a negative basophil response (Table 6) more likely to develop cardiovascular disease than those who show marked degranulation following a fatty meal?

In our experience, the basophil has proven to be a cell whose functional activity is reflected in its morphology. Our study has thus focussed on this process of degranulation rather than following the classical approach of counting the number of circulating basophils (Boseila[5]). The changes seen are entirely similar to those exhibited by the mast cell during its degranulation. To our knowledge the only other observations on degranulation in the basophil were made recently by Hunt and Hunt[16]. They studied the effect of systemic 48/80 on the basophil of the cockerel and found not only a drop in the basophil count, but also evidence of degranulation. The cells became smaller, had fewer granules and stained less intensely. It is noteworthy that the tissue mast cells showed no response.

We have attempted to quantitate the morphologic changes by doing a "basophil differential" count (Fig. 1) on a significant number of cells. Nevertheless, it must be recognized that the method is relatively insensitive and that

considerable secretion of granules may take place before it is obvious to the observer. It is for this reason that the rather massive feedings of fat (three-quarters of a pound) are necessary to afford conclusive proof that alimentary lipemia induces basophil degranulation. We are dealing with a physiologic stimulus which must be magnified to produce an obvious cellular response. Thus, we feel that probably all of the subjects show basophil secretion to some extent following a fatty meal, but that in only about half is it cytologically evident as degranulation to an unquestioned degree. Hence, here again, the individual trait, the genetic pattern, the age, and the sex all probably play a role in determining the extent of the basophil participation. Such individual variation makes consideration of mean values less forceful than the individual data, but nevertheless the means do faithfully follow the trend of continued slow degranulation.

It is important to realize that the overall morphologic state of the circulating basophil is not fixed, but may vary from day to day. Here is a cell intimately concerned with many phases of the allergic state (Chan and Yoffey[7]). It is not one solely concerned with fat transport. Our previous investigations have shown that a wide variety of allergens induce specific degranulation of this cell (Juhlin and Shelley[19,30]). It is presumably this cell's response which accounts for a large share of the anaphylactic reaction. Thus, we do not compare data gathered on different weeks, but rather must compare the changes of a few hours associated with a specific feeding. The experiments were all done at weekly intervals to allow adequate time for basophil regeneration, should depletion or depression of the cell occur as a result of any test procedure.

How does the fatty meal bring about the basophil degranulation reaction? There are many possible hypotheses. The first to occur to us was the possibility that the high fat intake induced a stress reaction and that the degranulation might reflect adrenal activity. The effect of cortisone in producing eosinopenia is well known, and Code, Mitchell and Kennedy[8] have shown cortisone to reduce the basophil count in man. Nevertheless we saw no degranulation of the basophils 3 hours after a single dose of 200 mg. of cortisone. A second possibility involves the allergic reaction. We have already demonstrated that such allergens as penicillin[30] will cause degranulation. Butter could well contain penicillin or other antigens, but our studies here have not revealed that the penicillin-sensitive individual has a unique response to the fatty meal. This possibility must be borne in mind, however, as a possible explanation for any unusually rapid explosive degranulation.

The most obvious mechanism for degranulation would involve the blood lipids. These are considerably increased in the subjects showing the greatest degree of degranulation. The triglyceride level reflects the chylomicron concentration and this appears to be a trigger factor. Levels of 8 mEq. per L. or above are associated more regularly with significant basophil degranulation. The exceptions bear further study, inasmuch as these subjects may have defective means of handling fats. The type of triglyceride does not seem to be a critical feature since butter and corn oil margarine gave identical results, yet chromatographic studies of the serum lipid pattern after feeding butter and corn oil (Ahrens *et al.*[2]) have shown great differences in the types of fatty acids found. A corn oil feeding may be associated with ten times as many di-unsaturated fatty acids as is a butter feeding. Again

looking to mechanisms, the nonesterified fatty acids could play a role, but we have no determinations of these. Also, soaps and bile salts might enter the picture and affect the basophil membrane.

On a theoretical basis, the most intriguing mechanism involves lecithinase A (phospholipase A, phosphatidase A). Högberg and Uvnäs[15] have shown this enzyme to be a remarkably potent agent for the degranulation of mast cells in the rat. Moreover, it has long been known to be a powerful component of snake and bee venom, acting on lecithin to produce the hemolytic compound, lysolecithin (Kates[20]). Recently, Zieve and Vogel[34] have brought this enzyme into new clinical prominence and established it as one of the enzymes circulating in the blood of man. Prior to that, the enzyme was known to be found in the pancreas and to participate in the digestion of fat in the intestine. Now its greater role in fat metabolism can be appreciated. Thus our studies, showing that the individuals with the most marked postprandial basophil degranulation also show an *in vitro* degranulation in the presence of lecithinase A, suggest that possibly this enzyme could be responsible for the degranulation normally associated with lipemia. Further studies are in progress.

Summary. We have found that postprandial lipemia is specifically associated with degranulation of the basophil. Such basophil degranulation is viewed as the mechanism for the physiological release of heparin and hence of importance in the clearing reaction. Furthermore, lecithinase A is viewed as possibly entering into this mechanism.

Since the basophil leukocyte is a delicate and rare cell, a special method has been employed for its handling. The method consists of fixation of 0.1 ml. venous blood in an acid alcohol fixative. This destroys the red cells and leaves the white cells suspended in a clear solution. These are then concentrated on a cellulose filter and the basophil specifically stained. The filter is mounted and forty consecutive basophils typed into distinct categories on the basis of size, staining properties and location of basophil granules.

A meal of butter (4 gm. per kg.) caused degranulation of the basophil leukocyte after 2 to 3 hours in about 75% of the subjects studied. Triglyceride levels of 8 mEq. per L. or above were regularly associated with significant basophil degranulation. No correlation was found with the degree of degranulation and phospholipid or cholesterol levels. Margarine, rich in unsaturated fatty acids, showed the same degranulation effect as butter. Meals consisting mainly of carbohydrates or proteins were without significant effect, as well as a single oral dose of cortisone (200 mg.).

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Photographs by Edward F. Glifort, Jr.

REFERENCES

1. Abell, L. L., Levy, B., Brodie, B. B., and Kendall, F. E.: J. Biol. Chem., **105**, 357, 1952.
2. Ahrens, E. H., Hirsch, J., Insull, W., Jr., and Peterson, M. L.: Dietary Fats and Human Serum Lipide Levels in Chemistry of Lipides as Related to Atherosclerosis: Page, I. H. (Ed.), Springfield, Illinois: Charles C Thomas, 1958.
3. Behrens, M., and Taubert, M.: Klin. Wchnschr., **30**, 76, 1952.

4. **Bessis, M.:** *in* The Cell: **Brachet,, J.,** and **Mirsky, A. E.** (Eds.). New York: Academic Press, Vol. 5, 1960.
5. **Boseila, A. A.:** The Basophil Leucocyte and Its Relationship to the Tissue Mast Cell. Copenhagen: Ejnar Munksgaard, 1959.
6. **Bouchier, I. A. D.,** and **Bronte-Stewart, B.:** Lancet, **1,** 363, 1961.
7. **Chan, B. S. T.,** and **Yoffey, J. M.:** Immunol., **3,** 237, 1960.
8. **Code, C. F., Mitchell, R. G.,** and **Kennedy, J. C.:** Proc. Staff Meet., Mayo Clinic, **29,** 200, 1954.
9. **Ende, N.,** and **Auditore, J. V.:** Nature, **189,** 593, 1961.
10. **Fredericks, R. E.,** and **Maloney, W. C.:** Blood, **14,** 571, 1959.
11. **French, J. E., Morris, B.,** and **Robinson, D. S.:** Brit. Med. Bull., **14,** 234, 1958.
12. **Fritz, I. B.:** Physiol. Rev., **41,** 52, 1961.
13. **Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A.,** and **Parish, H. H.:** Blood, **10,** 467, 1955.
14. **Hahn, P. F.:** Science, **98,** 19, 1943.
15. **Högberg, B.,** and **Uvnäs, B.:** Acta physiol. Scandinav., **48,** 133, 1960.
16. **Hunt, T. E.,** and **Hunt, E. A.:** Anat. Rec., **133,** 19, 1959.
17. **Jennings, M. A., Florey, H. W.,** and **Robinson, D. S.:** Quart. J. Exper. Physiol., **45,** 298, 1960.
18. **Jorpes, J. E.:** Heparin in the Treatment of Thromboses. An Account of Its Chemistry, Physiology and Application in Medicine. Oxford University Press, 1946.
19. **Juhlin, L.,** and **Shelley, W. B.:** J. Am. Med. Assn., in press.
20. **Kates, M.:** Lipolytic Enzymes, *in* Lipide Metabolism: **Bloch, K.** (Ed.), New York: John Wiley & Sons, Inc., 1960.
21. **Kelsall, M. A.,** and **Crabb, E. D.:** Lymphocytes and Mast Cells. Baltimore: The Williams & Wilkins Co., p. 192, 1959.
22. **Kern, F., Jr., Steinmann, L.,** and **Sanders, A. B.:** J. Lipid Research, **2,** 51, 1961.
23. **Korn, E. D.:** Lipoprotein Lipase *in* Chemistry of Lipides as Related to Atherosclerosis: **Page, I. H.** (Ed.), Springfield, Illinois: Charles C Thomas, 1958.
24. **Martin, H.,** and **Roka, L.:** Acta haemat., **10,** 26, 1953.
25. **O'Brien, J. R.:** Am. J. Med. Sci., **234,** 373, 1957.
26. **Olson, R. E.,** and **Vester, J. W.:** Physiol. Rev., **41,** 677, 1960.
27. **Reinhold, J. C.:** Method to be published in Standard Methods in Clinical Chemistry, Vol. 4, in press.
28. **Robinson, D. S.,** and **French, J. E.:** Pharm. Reviews., **12,** 241, 1960.
29. **Shelley, W. B.,** and **Florence, R.:** Unpublished data.
30. **Shelley, W. B.,** and **Juhlin, L.:** A New Test for Detecting Anaphylactic Sensitivity: The Basophil Reaction. Nature, in press.
31. Iidem: Functional Cytology of the Human Basophil in Allergic and Physiologic Reactions: Technique and Atlas, in preparation.
32. **Speirs, R. S.:** Ann. New York Acad. Sci., **59,** 706, 1955.
33. **Watson, W. C.:** Brit. J. Exper. Path., **42,** 15, 1961.
34. **Zieve, L.,** and **Vogel, W. C.:** J. Lab. & Clin. Med., **57,** 586, 1961.
35. **Zilversmit, D. B.,** and **Davis, A. K.:** J. Lab. & Clin. Med., **35,** 155, 1950.

(Interlingua on page 156)

UPPER GASTROINTESTINAL HEMORRHAGE DURING PREGNANCY

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Of the many devices Nature uses to protect the pregnant woman, relative immunity to upper gastrointestinal hemorrhage seems most remarkable to a gastroenterologist. It is known that temporary portal hypertension with esophageal varices may be a physiologic accompaniment of the last trimester of pregnancy (Palmer and Brick[2]) and that erosive esophagitis, with or without hiatus hernia, is a common complication of pregnancy (Scott and Deutsch[3]). Pernicious vomiting, as well as simple morning sickness, makes the group especially susceptible to the bleeding of Mallory-Weiss syndrome (cardial lacerations due to hard vomiting). Duodenal and gastric ulcer as possible threats are the exceptions, in that they ordinarily heal and cause little trouble during pregnancy. This, however, is not an invariable rule.

This report concerns experiences with 12 women who developed upper gastrointestinal hemorrhage during pregnancy. A precise diagnosis was made in each instance through use of the vigorous pandiagnostic approach during active bleeding. A thirteenth patient is included, although her hemorrhage was not personally witnessed, because of unusual and interesting sequels.

Diagnosis. In order to assure proper direction of emergency surgery or other manipulation for control of torrential upper gastrointestinal hemorrhage, should that become necessary, it has been found imperative, as well as safe and practical, to perform thorough diagnostic studies as soon as the bleeding patient is encountered (Palmer[1]). Esophagoscopy, gastroscopy and contrast fluoroscopy are carried out in quick succession, following ice-water lavage of the stomach. The lavage serves the dual purpose of furnishing emergency hemostasis and of clearing the region of blood. Precise diagnosis has been possible in about 90% of patients so managed, regularly with proper direction of emergency surgery when that was necessary.

When there is pregnancy, the pressures felt against emergency diagnostic manipulations may seem rather compelling, yet the potential dangers of hemorrhage in this situation are more pressing. This, at least, has been the philosophy behind management of the 12 cases discussed here. The technique of the vigorous diagnostic approach was followed, with the exception that the radiologic examination was omitted, because in every instance the bleeding lesion was located and identified endoscopically. This omission regularly proves a mistake under other circumstances, because a surgeon who operates in an emergency situation must if possible know beforehand, not only the bleeding lesion, but also all disease and abnormalities present in the upper gastrointestinal tract. In the present series, contrast fluoroscopy would have been carried out, in spite of the pregnancy, if endoscopic identification had not been made.

TABLE 1

No.	*Previous pregnancies*	*Month of pregnancy; bleeding lesion*	*Clinical Circumstances*	*Emergency measures; transfusion requirements, ml.*	*Outcome of pregnancy*	*Sequels*
1	2	9 Esophagitis	Hard vomiting 1st trimester and last week.	Support; 2000	Normal delivery during hemorrhage	1 week of odynophagia postpartum. Esophagoscopy after 1 month normal.
2	2	9 Esophagitis	Moderate morning sickness. Saint's triad and achlorhydria known.	Support; 1500	Normal	Postpartum pyrosis for 1 month.
3	1	9 Esophagitis (varices)	Uneventful pregnancy	Tamponade; 500	Normal delivery 4 days after hemorrhage	Onset dysphagia 1 week postpartum. Complete stricture in 3 weeks.
4	0	9 Esophagitis	Pernicious vomiting	Support; 2500	Normal	Onset dysphagia 6 days postpartum. Severe stricture in 2 months.

5	0	8 Esophagitis	Pernicious vomiting	Support; 3000	Normal	Onset dysphagia 3 years post-partum, stricture.
6	1	8 Varices (esophagitis)	Easy pregnancy	Tamponade; 2500	Normal	Esophagoscopy 5 weeks postpartum showed no varices.
7	4	5 Varices (esophagitis)	Easy pregnancy	Tamponade; 500	Normal	Esophagoscopy 4 weeks postpartum showed no varices.
8	0	7 Varices	A known cirrhotic. Easy pregnancy.	Tamponade; 4500	Normal	Varices persisted. Later portacaval shunt.
9	4	4 etc. Gastritis (hiatus hernia)	Moderate vomiting with small repeated hemorrhages beginning at 4th month.	Support; 1500	Stillbirth at 8½ months	None
10	0	3 Gastritis	Easy pregnancy.	Support; 1000	Normal twins	None
11	0	7 Mallory-Weiss	Hard vomiting 1st trimester and just before hemorrhage.	Support; 0	Normal	None
12	0	9 Mallory-Weiss (varices)	Hard vomiting week before delivery.	Support; 2000	Normal	None

OBSERVATIONS. Ten of the 12 patients were Caucasian, two Negro. The age at the time of hemorrhage ranged from 18 to 41 years, with a mean of 29 and an average also of 29 years. None of the patients had previously had recognized gastrointestinal bleeding.

The major features of the hemorrhages are outlined in Table 1. To be noted are the distribution among primiparas and multiparas and the chronologic point in the present pregnancy at which the hemorrhage occurred. The bleeding diseases were found to be subacute erosive esophagitis in 5 instances, esophageal varices in 3, acute erosive gastritis in 2, and the Mallory-Weiss syndrome in 2. One of the patients with gastritis also was found to have a hiatus hernia, a common accompaniment of both esophagitis and of pregnancy.

ETIOLOGY. As just mentioned, esophagitis is a common complication of pregnancy. The reason is not at all clear. It is probably a mistake to put much blame on the vomiting of pregnancy. Only 3 of the 5 patients in the present group had severe vomiting prior to bleeding, and one had no vomiting at all. Furthermore, as the table shows, bleeding from esophagitis came at the conclusion of pregnancy, when vomiting problems were much less important than they had been earlier. This same endoscopic and histopathologic form of esophagitis is encountered under a great many other clinical situations, and, as the commonest disease of the esophagus, it very often occurs by itself.

Erosive gastritis finds itself in almost the identical situation, etiologically speaking. It is an important source of hemorrhage under various circumstances, and it proved to be the explanation in 2 patients in the present series.

One patient who was bleeding from varices was known previously to have biopsy-proved cirrhosis. Her varices persisted after a normal delivery and she was treated by portacaval shunt 14 months later. The varices of the other 2 patients were proved esophagoscopically to have disappeared shortly after delivery, and liver biopsy and liver function tests following the puerperium were normal. The varices in these 2 patients undoubtedly represented the physiologic varices of pregnancy. Such varices have been encountered during pregnancy before (Palmer and Brick[2]), but bleeding has been observed in only these 2 patients. It is important to note that both of these patients also had esophagitis, for it is believed that varices bleed because they are eroded into from the mucosal surface.

The Mallory-Weiss syndrome accounted for the hemorrhage of 2 of the patients. This accident is a threat to anyone who vomits hard, yet, except for minor blood streaking of the vomitus, evidence of injury to the cardia by the vomiting of pregnancy is unusual.

TREATMENT. Emergency treatment was conservative in all cases, but support was aggressive. Ice-water lavage was relied upon heavily for emergency control of the bleeding, and it proved most effective. In addition, esophageal tamponade by Sengstaken tube was instituted as soon as bleeding varices were discovered. No special problems ascribable to the existence of pregnancy were encountered in carrying out tamponade. Tamponade was also used in one instance of bleeding esophagitis, when hemorrhage recommenced 2 days following ice-water lavage. The blood replacement requirements are shown in Table 1; because blood was always readily available and freely given as indicated, the figures offer a valid basis

for estimation of the severity of the hemorrhages.

OUTCOME AND SEQUELS. There were no fatalities. The pregnancies proceeded normally, except in one case. This patient had a small hemorrhage from erosive gastritis at 4 months. Thereafter, she had 6 other recognized bleedings, requiring altogether 3 blood transfusions of 500 ml. each. Death of the fetus was recognized 2 weeks prior to spontaneous delivery at 8½ months.

Sequels of significance were found only among the esophagitis group. Here the postpartum complications took the form not of further hemorrhage but of stricture. The experiences are outlined in the table. This tendency toward postpregnancy stricture is rather remarkable, in view of the common observation that only a small proportion of all people with subacute erosive esophagitis ever go on to develop significant fibrosis. Remarkable, too, was the swiftness with which obstructive symptoms developed. Dysphagia was felt by 2 of the patients within a week of delivery.

A thirteenth case illustrates the potential seriousness of the esophagitis sequels:

Case Report. CASE 13. This lady was first seen at the age of 72 years, when she was hospitalized because of massive hematemesis. Immediate ice-water lavage of the stomach stopped the hemorrhage, and esophagoscopy revealed that erosive esophagitis was responsible for the bleeding. In addition, there was a 7 mm. stricture at the junction of the middle and distal thirds of the esophagus. This prevented simultaneous gastroscopic examination. Meanwhile, 4000 ml. of whole blood were transfused. There was no further bleeding.

The history indicated that the patient's illness had actually begun 47 years previously, when, during her first pregnancy, she had had severe vomiting with hematemesis. Dysphagia began immediately following delivery and became severe thereafter. A course of esophageal dilatations for stricture was begun. It was found necessary to continue the dilatations, and after a while a dilator was given to the patient so she could carry out the procedure herself. She had passed a Fr. #22 Hurst bougie once every 3 weeks to the present time (about 800 dilatations). Meanwhile, she had 4 moderately severe hemorrhages.

Summary. Twelve women who developed upper gastrointestinal hemorrhage during pregnancy were managed by an aggressive diagnostic policy. The bleeding lesion in 5 instances was erosive esophagitis; in 3, esophageal varices; in 2, erosive gastritis; and in 2, the Mallory-Weiss syndrome. Therapy was conservative, without fatality. There was one stillbirth. Postpartum sequels were observed in the esophagitis group, in the form of rapidly progressive esophageal stricture.

REFERENCES

1. **Palmer, E. D.:** Diagnosis of Upper Gastrointestinal Hemorrhage. Springfield, Ill.: Charles C Thomas, p. 67, 1961.
2. **Palmer, E. D.,** and **Brick, I. B.:** J. Am. Geriat. Soc., **3,** 681, 1955.
3. **Scott, N. M., Jr.,** and **Deutsch, D. L.:** Am. J. Gastroenterol., **24,** 305, 1955.

SUMMARIO IN INTERLINGUA

Hemorrhagia Supero-Gastrointestinal in Pregnantia

Dece-duo feminas qui disveloppava hemorrhagia supero-gastrointestinal durante le pregnantia esseva subjicite a un aggressive tactica diagnostic. Le lesiones sanguinante esseva esophagitis erosive in 5 casos, varice esophagee in 3, gastritis erosive in 2, e syndrome Mallory-Weiss in le ultime 2. Le therapia esseva conservatori. Occurreva nulle morte. In un caso le infante nasceva morte. Sequellas post parto esseva observate in le gruppo a esophagitis in le forma de un rapidemente progressive strictura esophagee.

COMPARISON OF GUANETHIDINE AND GUANETHIDINE PLUS A THIAZIDE DIURETIC

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Guanethidine blocks the peripheral terminals of the sympathetic nerves and decreases tissue stores of norepinephrine (Maxwell *et al.*[14], Brodie and Kuntzman[2]). This agent has a hypotensive effect (Page and Dustan[16], Frohlich and Freis[9], Richardson and Wyso[17], Maronde *et al.*[13]). With dosages of guanethidine that did not produce disabling postural hypotension, an evaluation by the double-blind technique demonstrated no significant change of the supine systolic pressure and only a moderate fall in the supine diastolic pressure (Maronde *et al.*[13]).

In the present study, guanethidine and hydrochlorothiazide together produced a significant drop in both the supine and standing systolic and diastolic pressures. When guanethidine was given as the single active agent, it again was found that a significant fall in the supine systolic pressure did not occur, although there was a satisfactory drop of the standing pressures. This suggests a less important role of gravitational pooling of the blood in the production of the hypotensive effect, with the combined treatment.

Materials and Methods. The combinations of guanethidine and hydrochlorothiazide, guanethidine and placebo, and placebo and hydrochlorothiazide were compared in 27 patients with established essential hypertension. All had Keith-Wagener grades 1 and 2 fundi. They had attended the clinic for at least a 6-month period. Those who had congestive heart failure, elevated serum urea nitrogen or a history of malignant hypertension were not selected for the study. Fifty milligrams of hydrochlorothiazide and 15 mEq. of potassium were given 3 times daily during a 6-week control period. At the end of this time those patients with diastolic pressures of 110 mm. Hg or higher were selected for a 10-week double-blind study. They were placed into 3 groups by a random method. Numerical emphasis was placed on the guanethidine-hydrochlorothiazide group and statistical comparisons were made only on the corresponding pressures within groups. Sixteen patients were given guanethidine and the hydrochlorothiazide was continued; 6 patients were given guanethidine and a placebo identical in appearance to hydrochlorothiazide; 5 patients were given a placebo identical to the guanethidine and the hydrochlorothiazide was continued. All continued to receive the supplemental potassium. One observer, who did not participate in the evaluation of the blood pressure responses, dispensed the medications. The medicines were prescribed by the physicians who evaluated

the patients. They did not know whether active or placebo medication was dispensed. Twelve and one-half milligrams of guanethidine per day were prescribed initially. This was increased by weekly increments of 12.5 mg. until one of the following criteria was met: 1) the systolic pressure dropped below 150; 2) the diastolic pressure dropped below 90; 3) side effects made it necessary to stabilize or reduce the dosage.

The blood pressures were measured from the left arm while the patient was supine, then sitting and finally standing. The auscultatory method was used. All blood pressures were determined by 3 resident physicians who rotated after one clinic visit. The patients were seen every 2 weeks. In this fashion, one observer measured a patient's blood pressure every 6 weeks. The patients were supine for at least 10 minutes before the blood pressure was measured.

The averages and the values for t were determined, for statistical comparison, from the last 2 pressures of the 6 week control period and the last 3 pressures of the 10 week double-blind study.

TABLE 1.—MEAN BLOOD PRESSURE DURING A CONTROL PERIOD AND DURING DOUBLE-BLIND STUDY

	CONTROL *150 mg. Hydrochlorothiazide/day* *Blood pressures mm. Hg*		*DOUBLE-BLIND* *Guanethidine plus Hydrochlorothiazide* *Blood pressures mm. Hg*	
Patient	*Supine*	*Standing*	*Supine*	*Standing*
C.R.	190/120	160/120	153/86	151/91
H.G.	145/110	140/100	138/97	118/90
A.W.	140/105	160/120	122/88	102/71
J.B.	164/110	162/120	155/96	126/90
M.M.	200/140	200/110	197/105	183/91
G.R.	175/120	150/110	145/80	134/80
L.S.	190/130	190/130	153/90	142/91
L.L.	160/110	160/110	163/103	148/95
L.M.	180/96	168/110	170/94	161/102
J.A.	205/115	145/100	140/93	113/83
D.H.	175/130	170/130	158/110	145/108
B.J.	170/110	180/118	196/124	160/96
A.G.	210/120	200/140	191/113	180/103
I.A.	200/130	180/120	161/111	126/87
F.H.	160/120	160/120	162/113	146/111
C.S.	220/130	180/120	161/106	123/85
Average	180/119	169/117	160/100	141/93
			Guanethidine plus Placebo	
W.W.	260/180	260/180	213/131	180/121
I.C.	180/120	160/114	198/118	146/103
B.M.	165/115	150/115	148/96	133/94
W.L.	160/110	170/105	171/103	148/92
M.B.	230/120	228/122	190/103	150/95
V.D.	160/110	160/110	166/116	129/96
Average	192/125	188/124	181/111	147/100
			Hydrochlorothiazide plus Placebo	
C.P.	210/120	180/140	202/122	190/120
M.H.	200/116	190/120	202/113	193/118
I.K.	190/108	170/116	174/99	160/118
B.C.	185/120	140/110	200/116	144/104
J.B.	200/130	180/130	206/128	213/123
Average	197/118	172/123	200/115	180/117

Results. When guanethidine and hydrochlorothiazide were administered jointly the supine and standing systolic and diastolic pressures fell significantly ($p<0.001$). Guanethidine and the placebo resulted in a significant decrease in the standing systolic and diastolic pressures ($p<0.001$) and in the supine diastolic pressure ($p<0.02$). Hydrochlorothiazide and the placebo did not significantly alter the blood pressures from those of the control period (Table 1). The responses of the 2 groups of patients treated with guanethidine alone and with guanethidine and hydrochlorothiazide jointly are shown in Fig. 1. In the guanethidine-hydrochlorothiazide group, the average drop in blood pressures were 20 and 28 respectively for the supine and standing systolic pressures and 19 and 24 respectively for the supine and standing diastolic pressures. The mean fall in pressures for the group, in which guanethidine was the only active agent used, were 41 for the standing systolic and 14 and 24 for the supine and standing diastolic pressures (Table 1). These changes and their 80% confidence intervals are shown in Table 2.

TABLE 2.—FALL IN MEAN BLOOD PRESSURE, MM. HG, WITH 80% CONFIDENCE INTERVALS, WITH GUANETHIDINE PLUS HYDROCHLOROTHIAZIDE, AND GUANETHIDINE PLUS PLACEBO

	Systolic		*Diastolic*	
Group	*Supine*	*Standing*	*Supine*	*Standing*
Guanethidine + Hydrcholorothiazide	Degrees of freedom: 78			
	20 ± 6	28 ± 6	19 ± 3	24 ± 3
Guanethidine + Placebo	Degrees of freedom: 28			
	Change not significant	41 ± 7	14 ± 4	24 ± 4

At the end of the double-blind study, the average dose of guanethidine, when used as the sole agent, was 60 mg. (range 25 to 125) but the average dose was 37 mg. (range 12.5 to 75) when it was given in conjunction with hydrochlorothiazide. No significant difference in the frequency of side effects was apparent.

Discussion. In a previous study, guanethidine, in amounts that were well tolerated, did not significantly change the supine systolic pressure. A significant drop did occur in the supine diastolic, standing systolic and standing diastolic pressures (Maronde *et al.*[13]). In the present study, this was confirmed and it was demonstrated that hydrochlorothiazide augmented the hypotensive effect of guanethidine. There was an effect upon the supine pressures with the combined therapy not present with either guanethidine or hydrochlorothiazide singly.

The hypotensive actions of guanethidine and hydrochlorothiazide apparently differ in their origins. The mechanism of the hypotensive effect of the thiazide is uncertain. It has been suggested that a decrease of sodium within the arteriolar wall, after thiazide administration, decreases the peripheral resistance (Aleksandrow, Wyaznacka and Gajewski[1]). The sodium ion also has been implicated in the contractility of the arterioles (Haddy[10], Friedman,

Jamieson and Friedman[8]). An enzyme system, activated by normal concentrations of sodium ion within the arteriolar wall, but inhibited by high levels and made less active by low concentrations, has been postulated as influencing this contractility (Mendlowitz constant an increase in the sodium content (Daniel and Dawkins[3], Freed, St. George and Rosenman[5]). In addition to these changes in arterial wall sodium content, the hypotensive action of the thiazides has been related to a decreased cardiac output and plasma

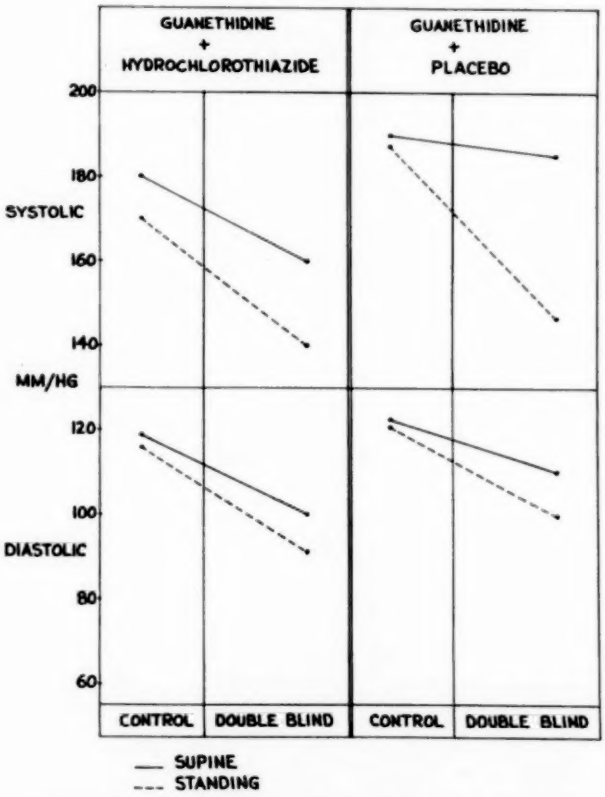

Fig. 1.—Mean pressures during the control and double-blind periods of the guanethidine-hydrochlorothiazide and guanethidine plus placebo groups.

et al.[15]). Thiazide, therefore, by depleting arteriolar sodium theoretically could result in a decreased contractility (Mendlowitz *et al.*[15]). An increased sodium content of arterial walls of animals made hypertensive by different experimental means has been reported (Tobian and Binion[18]). Some investigators have not found as marked or as volume (Freis *et al.*[7], Dustan *et al.*[4]). A more specific effect is suggested, however, as correction of the plasma volume does not bring the blood pressure up to control levels and the long term hypotensive effect may occur without a measurable decrease in plasma volume (Hollander, Chobanian and Wilkins[12]).

Guanethidine, by blocking the peripheral terminals of the sympathetic nerves, decreases peripheral resistance and decreases tissue stores of norepinephrine (Maxwell *et al.*[14], Brodie and Kuntzman[2], Richardson and Wyso[17]). As with the ganglion blocking agents, postural venous pooling of blood is an important factor in the production of the hypotensive effect (Richardson and Wyso[17], Freis *et al.*[6]). Norepinephrine is required for the normal compensatory venoconstriction that occurs in the standing position (Hickler *et al.*[11]), and guanethidine interferes with this mechanism (Richardson and Wyso[17]). Because of the postural nature of the venous pooling, a satisfactory hypotensive response while in the supine position often is associated with disabling hypotension while standing. In the present study, the dosage of guanethidine was regulated by the occurrence of side effects and the blood pressure levels (see **Methods**). When hydrochlorothiazide was given in addition to guanethidine, our observations revealed a significant fall in the supine pressures with less guanethidine and without an increased incidence of disabling postural hypotension. This suggests the smaller amounts of guanethidine required when thiazides are given concomitantly lower the supine pressures without marked impairment of the venoconstrictive reflex. Therefore, a drop in the supine blood pressure of the group treated with guanethidine and hydrochlorothiazide could be induced without an increased incidence of postural hypotension. This may be related to a less complete depletion of the tissue norepinephrine by the smaller amounts of guanethidine.

Summary. Guanethidine, when given singly in amounts sufficient to result in a satisfactory fall in the standing blood pressure, did not produce a significant change in the supine systolic pressures and only a moderate fall of the supine diastolic pressures. Guanethidine and hydrochlorothiazide, when administered together, resulted in a significant fall in both supine and standing blood pressures. Augmentation of guanethidine by hydrochlorothiazide was demonstrated. When these agents were given together it is likely that gravitational pooling of the blood was less of a factor in the production of the hypotensive effect. Thiazide diuretics most likely augment the hypotensive action of guanethidine as the result of the decreased vascular reactivity to norepinephrine. The lower dosages of guanethidine required with concomitant thiazide administrations produced a satisfactory fall in the standing blood pressures and also reduced the supine pressures significantly. This drop in supine pressures, without an increased incidence of postural hypotension, suggests that the venoconstrictive reflex is impaired to a greater degree when larger amounts of guanethidine, given alone, produce comparable changes in the standing pressures, possibly as the result of more complete depletion of tissue norepinephrine.

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REFERENCES

1. **Aleksandrow, D., Wyaznacka, W., and Gajewski, J.:** New England J. Med., **260**, 51, 1959.

2. **Brodie, B. B.**, and **Kuntzman, R.**: Ann. New York Acad. Sci., **88,** 939, 1960.
3. **Daniel, E.**, and **Dawkins, O.**: Am. J. Physiol., **190,** 71, 1957.
4. **Dustan, H. P., Cumming, C. R., Corcoran, A. C.,** and **Page, I. H.**: Circulation, **19,** 360, 1959.
5. **Freed, S. C., St. George, S.,** and **Rosenman, R. H.**: Circulation Res., **7,** 219, 1959.
6. **Freis, E. D., Rose, J. C., Partenope, E. A., Higgins, T. F., Kelley, R. T., Schnaper, H. W.,** and **Johnson, R. L.**: J. Clin. Invest., **32,** 1285, 1953.
7. **Freis, E. D., Wanko, A., Wilson, J. M.,** and **Parrish, A. E.**: J. Am. Med. Assn., **166,** 137, 1958.
8. **Friedman, S. M., Jamieson, J. O.,** and **Friedman, C. L.**: Circulation Res., **7,** 44, 1959.
9. **Frohlich, E. D.,** and **Freis, E. D.**: Med. Ann. Dist. Columbia, **28,** 419, 1959.
10. **Haddy, F. J.**: Proc. Council for High Blood Pressure Research, Am. Heart Assn., Hypertension, **8,** 75, 1959.
11. **Hickler, R. B., Wells, R. E., Jr., Tyler, H. R.,** and **Hamlin, J. T., Jr.**: Am. J. Med., **26,** 410, 1959.
12. **Hollander, W., Chobanian, A. V.,** and **Wilkins, R. W.**: Ann. New York Acad. Sci., **88,** 975, 1960.
13. **Maronde, R. F., Barbour, B., Haywood, L. J.,** and **Denney, J.**: Ibid., **88,** 990, 1960.
14. **Maxwell, R. A., Plummer, A. J., Schneider, F., Polvaski, H.,** and **Daniel, J. J.**: J. Pharmacol. & Exper. Therap., **128,** 22, 1960.
15. **Mendlowitz, M., Naftchi, N., Gitlow, S., Weinreb, H., L.,** and **Wolf, R. L.**: Ann. New York Acad. Sci., **88,** 964, 1960.
16. **Page, I. H.,** and **Dustan, H. P.**: J. Am. Med. Assn., **170,** 1265, 1959.
17. **Richardson, D. W.,** and **Wyso, E. M.**: Ann. New York Acad. Sci., **88,** 944, 1960.
18. **Tobian, L., Jr.,** and **Binion, J.**: J. Clin. Invest., **33,** 1407, 1954.

SUMMARIO IN INTERLINGUA

Comparation de Guanethidina Sol con Guanethidina in Combination con un Diuretico Thiazidic

Guanethidina, administrate sol in quantitates sufficiente a resultar in un satisfacente reduction del tension de sanguine orthostatic, non produceva un alteration significative del tensiones systolic in decubito dorsal e solmente un moderate reduction del tensiones diastolic in decubito dorsal. Guanethidina e hydrochlorothiazida, quando administrate insimul, resultava in un declino significative del tension sanguinee tanto in decubito dorsal como etiam in position erecte. Potentiation de guanethidina per hydrochlorothazida esseva demonstrate. Quando iste duo agentes esseva administrate insimul, il es probabile que le thesaurisation gravitational del sanguine esseva un factor minus importante in le production del effecto hypotensive. Diureticos thiazidic augmenta probabilissimemente le action hypotensive de guanethidina como resultato del reducite reactivitate vascular a norepinephrina. Le plus basse doses de guanethidina requirite in administration concomitante con thiazida produceva un satisfacente declino del tensiones orthostatic de sanguine e etiam reduceva significativemente le tensiones in decubito dorsal. Iste declino in le tensiones in decubito dorsal, non accompaniate de un plus alte incidentia de hypotension postural, suggere que le reflexo venoconstrictice es disturbate plus marcatemente quando plus grande doses de guanethidina, administrate sol, produce comparabile alterationes in le tensiones orthostatic, possibilemente como resultato de un plus complete depletion del norepinephrina tissular.

PROGRESS
OF
MEDICAL SCIENCE

OPHTHALMOLOGY

UNDER THE CHARGE OF

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OCULAR SENESCENCE

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THE main difficulty in identifying aging processes is that induced changes occur in the body and its tissues, including the eye, throughout life and interfere with the predetermined course of metabolism. The metabolism of man is connected with oxygen consumption. The less oxygen is supplied to the tissue, the less intensive is the metabolism. According to this principle tissues can be divided into those with low oxygen metabolism, or bradytrophic tissues, and those with high oxygen metabolism, or tachytrophic tissues. The cornea, the lens, the sclera, and certain layers of the blood vessels are examples of tissues of low oxygen consumption. They have a common fate: with increasing age their water content decreases. This desiccation process leads to densification, which is chemically characterized by increase of the protein content. This densification makes the exchange of nutrients and the transport of waste more difficult.

The senile modifications usually found in the eyes of people past the 5th decade which assume special characteristics for each ocular tissue belong to some processes of a more specific nature: arteriosclerosis, fatty degeneration, calcareous infiltration, increase in collagen tissue and hyaline degeneration. While these changes are frequently found in older eyes, the finding of one or more of them in eyes of younger persons rules out the possibility that they are inevitable and pathognomonic of senility. The finding of a given senile lesion does not permit any conclusion on the actual age of the subject. One cannot disregard the time element in ocular vulnerability to induced changes of vascular disease, diabetes, paralysis, anemia, malignancy and a host of terminal diseases that are most often found in the eyes of the elderly simply because they

lived long enough to induce a greater exposure to chance afflictions. Senescence, therefore, is the result of all things that can occur in a life time beyond the average span.

THE EYE IN OLD AGE. The eye in old age suffers along with the rest of the body the changes in tissue and vitality that result from retarded metabolism of oxygen and replacement of waste products in the exchange of nutriment. That all the changed characteristics attributed to old age are due to the same influences is untenable even though the biochemical processes are similar.

THE ORBIT. Longevity is the reward for physical fitness. The optimal weight for age and height as calculated by biostatisticians is a function of surveys at intervals and modified by economic conditions. As the population increases in the over-65 bracket, the physical status of the elderly is correspondingly prolonged in the presenile category, the evidences of degeneration and decline postponed and the historic gouty and crippled oldsters are rare. When growth and energy requirements of the young and middle aged are no longer necessary to maintain the rate of metabolic change in the body, the dietary habits of the individual also must be changed to avoid excess weight. When economic conditions permit a wide variety of food, the overweight of the rich and the emaciation of the poor are brought into line with an over-all average and no longer mark the elderly as pictured in the past. The bulging eyes of the overweight and the sunken eyes of the undernourished are not the result of age but of disease or dietary indiscretions, or both. By actual count, the majority of elderly people are more likely to be underweight than overweight, having escaped the systemic disorders that so often accompany excess fat and contribute to cardiovascular disease.

The facial expression of senescence is due to changes in the skin and subcutaneous structures, muscles, fascia and bone. Sunken or bulging eyes reflect incidental and extraneous deviations in growth and development rather than the inevitable degeneration of age. There is a characteristic distribution of fat in the body including the face that characterizes the elderly together with skin wrinkling and altered muscular movement and loss of teeth. Such changes may be attributed to senescence.

The bones of the orbit of elderly (over 65) persons are eburnated, but unchanged in form after reaching adult growth. Ocular muscles become fibrous and lose some of the fluids that give form and substance. Alterations in the distribution of fat within the orbit are more evident than the total loss except in cases of bodily weight loss. Here, again, the relative loss of fat in the orbit and general weight loss may not be proportional. The soft tissue content of the orbit other than fat undergoes little change; the vascular system, the nerves and fascia show qualitative rather than quantitative variation in the senescent phase.

The lacrimal apparatus and even the extraocular muscles are subject to physiological involutional changes and to the degenerative and pathological phenomena which supervene with the advent of time. Degenerative changes represent some of the most typical lesions of the lid. The commonest of these is senile degeneration of the lid, causing folds and redundancy by loss of tone and elasticity. "Pouches and bags" may originate from tissue edema or from loss of tone and consequent protrusion of orbital fat.

CONJUNCTIVA. In senile individuals, atrophy of the conjunctiva affects chiefly the subepithelial connective tissue. Transparency is reduced by increased thickness of the epithelium and

keratinization. It gradually assumes a yellowish color with prominence of small blood vessels, particularly about the limbus, and scattered pigmentation. In the canthi, folds often appear as redundant membrane, and near the limbus proliferation of epithelial and hyaline deposits form pingueculae in the palpebral fissure.

Although protected by the eyelids, the conjunctiva and cornea are exposed to trauma and infection with inflammatory reactions and often tissue destruction. A remarkably rapid repair follows non-inflammatory injury when the scant blood supply is considered. Healing of wounds of the conjunctiva is expedited by an out-pouring of serum and blood that forms a fibrin clot which closes the edge of the wound. Healing of the conjunctiva from clean wounds or inflammatory disease results in scars of fibrous tissue with disappearance of elastic tissue fibers and increased thickness with calcareous deposits. Obviously the conjunctiva is more affected by processes of disease and repair than by retarded growth and development so that age alone has no pathognomonic indication. However, the almost universal occurrence of altered conjunctiva from frequent attacks of inflammation found in the aged permits the symptomatic description as senescent because the thin clear transparence of youthful conjunctiva is never found in the aged. Undoubtedly all changes in the conjunctiva cannot be ascribed to either age or extraneous damage. The healing power of the conjunctiva is not seriously impaired in elderly persons so that surgical procedures are not more hazardous in the aged. In arteriosclerotic persons the blood vessels of the conjunctiva are usually either long straight unkinked arteriole accompanied by a parallel venule, traversing much of the length of the temporal bulbar conjunctiva or a conspicuous group of parallel straight segments of such vessels. This picture is often associated with an arteriosclerotic fundus and uncommonly with a hypertensive fundus and seems to be the counterpart in the conjunctiva of the findings of an arteriosclerotic fundus.

CORNEA. The undamaged cornea retains its normal translucency throughout life although histologic examinations reveal slight structural changes in epithelium and endothelium that appear after 45 years of age. The more prominent changes appear at the margins of the cornea. The conjunctival limbus encroaches on the cornea with vascularization and surface irregularities most evident on either side of the cornea. Pingueculae and pterygia destroy or replace normal corneal epithelium where encroachment occurs resulting in flattening and changes in corneal astigmatism. Corneal sensitivity decreases rather rapidly after the age of 50 and reaches a minimum in old age.

The substantia of the cornea is infiltrated by fine lipoid deposits in the peripheral zone that give a gray ring-shaped opacity just inside the limbus, known as the arcus senilis or arcus lipoidis. "These deposits usually cannot be recognized in sections stained with hematoxylin and eosin, although sometimes foamy lipoid histiocytes are seen in the limbal tissue; but they are demonstrable with Sudan III or any of the other fat stains"[5]. Arcus senilis is thought to be associated with changes of age and atherosclerosis but it is also seen in younger individuals and at times induced by local irritation and inflammation.

Small excresences (Henle warts) of hyalin are formed on the posterior surface of Descemet's membrane usually near the periphery in the eyes of old persons. In the most seriously in-

volved cases the lipoid infiltration of Descemet's membrane involves the central corneal zone where they are observed in ophthalmoscopic examination as guttate endothelial dystrophy. Unpigmented lines in the senile cornea are caused by ruptures in Bowman's membrane, said to be due to inverse astigmatism known to develop in old age.

Generally diminished sensitivity of the cornea occurs especially at the periphery in subjects whose cornea is clinically normal. In subjects whose corneas present a senile alteration such as gerontoxin, limbic sclerosis, Hassall-Henle's corpuscles, cornea gutta, cornea farinata, and others, the first three alterations bring about a considerable reduction of the sensitivity. This reduction is directly proportional to the degree of the alterations and to their topographical situation. While endothelial and epithelial dystrophy are considered generally as degenerative diseases due to old age, similar dystrophies are occasionally found in young persons which points to familial and hereditary tendencies.

SCLERA. That scleral rigidity increases with age is shown by the fact that scleral tonometric measurements in the elderly are higher than corneal ones. Calcium and fat deposits are sometimes seen in the sclera and may be demonstrated by special staining methods. Hyalin and calcium plaques may be seen clinically as slate gray patches located near the limbus, anterior to the insertions of the internal and external rectus muscles. On the inner surface of the sclera, fibers arising near the Descemet's membrane, partly scleral and partly uveal, form the trabecula. Ashton did not regard sclerosis of the trabecula as an aging process in an individual without glaucoma. Depending on what is meant by sclerosis of the trabecula he states that, "In chronic glaucoma, it is a fibrous replacement, which is certainly not a senile change"[1].

The pores of the trabecula are continuous with drainage spaces of Schlems' canal. Those at the surface of the sclera are called aqueous veins. These veins are said to be larger in older persons, probably due to increased scleral rigidity and shrinkage of scleral fibers. That such changes are senile rather than brought about by glaucoma and other extraneous factors is not generally admitted.

UVEA. The uvea, including the iris, ciliary body and choroid, undergoes a variety of changes that are generally found in the eyes of the aged but the character and extent of such changes do not permit a clear differentiation between disease processes and senile degeneration. A small pupil, with depigmentation of the pupillary margin, is commonly seen in old people. The pupillary zone undergoes degenerative change and the sphincter itself becomes atrophic, giving the appearance of a frayed discolored iris. The epithelium becomes alternately atrophic and proliferated. Degeneration of the pigment epithelium of the iris may result in release of melanin granules or the casting off of complete cells or groups that become attached to the posterior surface of the cornea, usually taking the form of a vertically placed spindle (Krukenberg's pigment spindle).

In the iris senile changes include depigmentation of the pupillary margin, fibrosis and hyalinization of the sphincter muscle, fibrotic thickening of the vascular adventitia and increase in density of the iris stroma with partial obliteration of the iris crypts. No specific relation of any of these phenomena to general or local atherosclerosis has been established.

The ciliary body atrophies with age. The processes become fibrotic and

hyalinized with consequent obliteration of blood vessels and diminished power of the contractile fibers. The ciliary muscle of aged eyes is often atrophic and shows interstitial fibrosis. Here, too, the pigment epithelium exfoliates carrying melanin to the interstices between the processes and into the posterior aqueous. Loose pigment cells are found in the chamber angle where they may presumably tend to diminish aqueous flow through the trabecula giving rise to increased intraocular tension. The chamber angle is narrower in old age, the anterior and posterior aqueous chamber is shallower as age increases. The higher intraocular pressure characteristic of age may be attributed to an extent by the structural changes at the chamber angle.

AQUEOUS SECRETION. Tonograms on a total of 909 normal eyes were analyzed by Becker[2]. These were divided into three age groups: 1) those 40 years of age or less, 2) those between 41 and 60, and 3) those patients over 60 years of age. There was little variation in intraocular pressure with age, averaging 16.1 $\pm$ 2.8 (S.D.) for the entire series. However, outflow facility declined steadily with age and differences among the three age groups were statistically significant. The average data further subdivided by decade for pressure, outflow facility, and rate of secretion show even more evidently that though pressure remains reasonably constant, outflow facility and the rate of secretion declines steadily with age, and the rate of secretion presents a rapid fall off after the age of 60 years.

In a similar series of 910 eyes with proved simple glaucoma, the decline in aqueous secretion after the age of 60 is also highly significant. A series of 54 enucleated normal eyes were perfused. The outflow facilities showed a similar decrease in average facility with age, much as in the tonographic data.

The most consistent tonographic finding in these series of eyes, both glaucomatous and normal, is a marked decline in the rate of secretion of aqueous humor after the age of 60 years. This correlates well with the histologic picture in the ciliary body. An experienced eye pathologist can estimate the age of the patient from the degree of atrophy of the ciliary processes. A greater atrophy and hyalinization of ciliary processes occurs in glaucomatous eyes, corresponding to the lower average secretory rate found.

On the other hand, it has been demonstrated that a decrease in outflow facility may fail to produce a rise in pressure because of a reflex compensatory decrease in secretory rate. Such normalization of intraocular pressure in spite of impaired outflow facility is a common finding in glaucomatous eyes. Whatever its mechanism, it is important to emphasize that the decrease in the rate of secretion of aqueous humor with age tends to prevent glaucomatous pressure rises and glaucomatous damage to the optic nerve. Physiologic hyposecretion may account for the relatively infrequent occurrence of pressure elevations in spite of the comparatively high incidence found in eyes of the aged.

Blood circulation of the uvea is altered by processes that afflict the blood vessels themselves or the cellular tissue through which they course either by disease or degeneration. The ciliary processes undergo hyaline degeneration with consequent hardening and shrinking (sclerosis).

The structure of the choroid is less specialized than the anterior uvea but it, too, suffers similar atrophic degeneration most noticeable in the periphery of the fundus and the peripapillary region associated with and perhaps by fibrosis, hyalinization and occlusion of choroidal vessels, particularly the

choriocapillaries. The vascular system of the uvea is subject to degenerative changes similar to the vascular tree of the retina. This discussion of the changes attributed to aging processes of the arteries and arterioles in both tissues will be elaborated under consideration of the retina.

The choroidal arteries are extremely prone to atherosclerotic lesions; in fact, atheromatous plaques are to be found in the larger choroidal arteries near the posterior pole of the eyeball in almost every case of advanced generalized atherosclerosis and the extent and frequencies of these lesions suggest that occlusions of the affected arteries are more common than atherosclerotic occlusions of the retinal artery. Nevertheless, infarct and apoplexy of the choroid are extremely rare. Atherosclerotic choroidal hemorrhages do occur and may on occasion be quite massive. Presumably the collateral anastomotic connections in the choroidal arterial tree are sufficiently abundant to protect the choroid from major damage as the result of an isolated arterial occlusion. Fibrosis and hyalinization of the areolar interstitial tissue of the ciliary processes have no specific relation to local vascular sclerosis.

The ocular changes most properly attributed to senescence are, in order of frequency, cataract, macular degeneration and glaucoma. The latter two are discussed elsewhere.

LENS. Senile cataract is of two forms: the nuclear or sclerotic, and the cortical or liquefactive; both may be present in a single case. Since the sclerosing process is a normal manifestation of advancing age, its development in aggravated form in cataract of the sclerotic type is properly to be regarded merely as an early or exaggerated local senile manifestation. It is to be presumed that every one who lived to a sufficiently advanced age would develop this form of cataract.

The autolytic processes that play a part in the cortical cataract seldom take place simultaneously throughout the whole lens cortex. Progress is intermittent and irregular. Finally, if the whole of the cortex is liquefied, the nucleus sinks downward within the sac of milky fluid (Morgagnian cataract). The cause of the lenticular changes are sought in the realm of chemistry as the lens tissue is known to be highly resistant to radical metabolic influences that are so noticeable in other parts of the body, particularly lipoid degeneration. The subcapsular vacuoles arising in the axial part of the lens are characteristic of old age, although their significance is rarely recognized, mainly because they cannot be seen except by means of a slit lamp. Symptoms of increasing vacuolation are slight blurring of the outline of objects seen, colored halo surrounding lights and inability to do fine work, a gradual diminution in visual acuity. The symptoms of lens degeneration may be attributed to alteration in the carbohydrate metabolism, its principal source of energy, since the lens relies on carbohydrate for its nutrition. Changes in the epithelial capsule of the lens are more frequent in older persons and the nutritional element is more significant as the eye gets older. There is no evidence that the cataracts are in any specific sense due to use of the eyes, but prolonged exposure to bright light in tropical areas and in certain industries may be considered a contributing factor when applied over a long time.

The variation in visual acuity in succeeding age decades is given by Kornzweig, Feldstein and Schneider for a group of men and women in the New York area. Figures from a similar group in another nation may show some differences but in the main they indicate senile tendencies (Table 1)[7].

FUNDUS. Examination of the eye with the biomicroscope and the ophthal-

TABLE 1.—VARIATION OF VISUAL ACUITY IN SUCCEEDING AGE DECADES

	Age (yr.)							
	60–69		*70–79*		*80–90*		*90 and over*	
Visual Estimate	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1. Good	228	81.64	859	78.23	418	56.5	18	39.2
2. Fair	16	5.99	122	11.11	164	23.1	13	28.2
3. Poor	7	2.62	45	4.08	65	9.4	8	17.4
4. Very poor	11	4.12	55	5.01	69	9.7	6	13.1
5. No light perc.	5	1.8	17	1.55	9	1.3	1	2.1
Totals	267 eyes		1,098 eyes		725 eyes		46 eyes	

Cataract formation is definitely one of the concomitants of old age. All statistics so far have shown that it is inevitable if one lives long enough. However, when the figures are examined from the point of view of visual function it is surprising how relatively few individuals are sufficiently handicapped visually to require surgical correction.

moscope reveals changes in the ocular media which result from injury, disease and involution. The transparency of the media determines to a large extent the distinctness of the fundus picture. When the media are clear as in normal eyes of young persons, the details of the fundus picture are clearly seen over more than one-half of its extent magnified three to six times by the lenses of the ophthalmoscope.

The background of the normal fundus is pigmented throughout with usually a deeper concentration in the macular area and in the circumpapillary zone. The inner layer of the choroid, the choriocapillaries, appears without landmarks except in some instances of light pigmentation the larger vessels of the choroid are visible in outline.

The principal anatomical features of the fundus which are visible with the ophthalmoscope and which suffer the most significant changes as the result of accident and disease are the optic disc and the retinal vascular tree.

The size and shape of the disc varies with the size and shape of the eyeball. Considerable variation in the shape of the disc from round to oval in normal eyes may be due to irregularities in the refractive media through which it is seen, but the disc is always larger in structurally large myopic eyes and small in highly hypermetropic eyes. The disc in highly myopic eyes may be expanded to double or triple the normal size by stretching of the sclera and retraction of the choroid. As the disc is entirely scleral, the margins are usually sharply outlined and visible but may be entirely hidden by the optic nerve and blood vessels that pass through the cribriform plate. The transparency of the nerve at the disc margin may be dimmed by glial proliferation and unabsorbed portions of nerve sheaths.

The central artery branches over the disc in a familiar pattern, with branches serving the four quadrants of the fundus and without anastomosis. The branches are different in structure from the central artery and are known as arterioles. The venous tree is similar to the arteriolar system throughout the fundus. Vascular accidents are suffered in either the arterial or venous system and the retinal blood supply is subject to significant changes as a result of changes in the blood vessels. The

clear view of blood vessels in a living organ by ophthalmoscopy is a valuable diagnostic aid, as the vascular system throughout the body is subject to influences of disease and involutionary tendencies that are reflected in tissue response.

Familiar alterations in the vascular system of the retina are attributed to involutionary attrition. In many respects they resemble the changes that result from vascular degeneration induced by disease. The designation arteriosclerosis has been used to describe the most common change in the vascular system and clinical descriptions of fundus pictures seldom make any more specific reference to the state of the vessels. Changes in the fundus commonly seen in the eyes of older people may be influenced by accident, disease or faulty development but fundamentally they are results of nutritional deficiencies and the tendencies to inclusion of lipoid cells between normal cells and fibers. Such are the changes at the periphery of the retina. Cystoid spaces occur in middle life and increase in number and size as age progresses. Fundamentally, the involutionary changes in the arterial system are reflected in secondary changes in the retina and choroid. As oxygen is the prime requirement for vitality of the retinal sensory cells any alteration in retinal circulation is significant. Interruption of the blood flow in the central artery of the retina for 5 minutes may be followed by complete blindness; a branch of the central artery closed for 5 minutes may result in permanent loss of vision in the portion of the retina supplied by the affected arteriole. On the venous side, closure of a vessel results in edema, congestion and hemorrhages in the retina and even into the vitreous. Most of the serious diseases in the ocular fundus are associated with vascular changes directly or indirectly.

Arterial disease has been carefully studied for several centuries. The atheromatous lesion was observed first. Atheroma occurs in arteries of all sizes; it is a patchy, subendothelial, fatty, degenerative lesion with associated fibrosis. It occurs with or without high blood pressure and its origin is not known. "Later, the name arteriosclerosis was coined to describe a diffuse hardening or stiffening of the arteries, contrasting with patchy atheroma. The common type is that which is due to high blood pressure." . . . "Decrescent or involutionary sclerosis is defined as an aging process affecting arteries and arterioles, appearing histologically as a relative or replacement fibrosis of patchy distribution. The condition is not caused by, but may be associated with, high blood pressure"[8].

Ophthalmologists have been prone to speak of "retinal arteriosclerosis" regardless of the size of the vessels involved. Some, more discriminatory, have used the term "arteriolosclerosis" in reference to the changes in the branches of the central retinal artery. That the retinal arterial vessels are visibly changed in vascular disease and in association with high blood pressure and systemic disorders (diabetes) is not denied, but there is disagreement in the order of the changes seen in the retina with the ophthalmoscope, whether the hypertension precedes and results in vessel changes or the other way around. No other organ of the body affords the visualization of tissue changes as are seen in the ocular fundus so comparisons cannot be made. Consequently, the comparisons must be made by microscopy. In general it may be said that similar changes in advanced vascular sclerosis are found in arteries and in arterioles except for the natural basic structure of the ves-

sel walls. In Elwyn's[4] opinion, arteriosclerosis is one and the same disease, whether the intima presents an atheroma, a localized fibrous thickening, or a hyaline thickening of the arterioles. He thinks that the changes due to aging of the arteries must be separated from the changes which constitute arteriosclerosis proper. Aging manifests itself in dilatation and tortuosity of the arteries. When the irregular and patchy changes of arteriosclerosis are added, they produce an irregularity in the appearance of the blood column. There will be angular tortuosity of the vessel wall, localized variations in the caliber of the arteries, localized arteriovenous constriction and widening of the arterial reflex. These signs, he states, will become noticeable ordinarily only in the 6th decade and beyond.

The presence of signs of retinal arteriosclerosis in young or middle-aged persons suggests persistent arterial hypertension, but the signs are not the signs of general arterial hypertension but of aging and arteriosclerosis. In the stages of essential hypertension and sometimes even in advanced stages there are, according to Elwyn, no changes in the retinal vessels to indicate premature aging or arteriosclerosis. In long-standing cases of hypertension, signs of aging and sclerosis may appear and some of the arteries assume a copper-wire or silver-wire appearance indicative of arteriosclerosis.

Leishman[8] applied the interesting concept of "defense by sclerosis" to the problem of determining the significance of retinal arteriolar lesions in the diagnosis and prognosis of systemic hypertension. He wrote: "This concept of defense by sclerosis is conceived in the limited sense that the reactive changes induced in a vessel wall as a result of hypertension appear to protect the wall from the more severe acute degenerative lesions of high blood pressure. Sclerosis may not only protect the wall from necrosis, but, also, may in some cases predetermine, and in others modify, the whole course of the hypertensive disorder." Leishman thought that, if hypertension occurs in a person whose vessels are significantly affected by involutionary sclerosis, he will be protected from serious elevations of diastolic pressure in proportion to the degree and extent of his sclerosis, because of the reduced areas capable of effective hypertonus or diffuse active constriction. When, or if, the blood pressure rises, only those portions of senile arterioles will become hypertonic which are not fibrosed and which are still contractile. Other portions will dilate passively. If the whole vessel is involved by fibrosis, it will dilate throughout its length. Then, even if the hypertension is severe, senile ("old") retinal arterioles will be larger and wider than normal. Young retinal arterioles, on the contrary, will react to hypertension by diffuse hypertonus (contraction, narrowing) throughout their length. Histologically, "old" vessels will have normal walls in the hypertonic segments and thinner than normal walls in the dilated segments with more collagen and fewer nuclei than normal; diffusely hypertonic "young" arterioles will have normal walls. Involutionary sclerosis is normal in old age but may occur prematurely in arterioles as well as in the larger arteries. For this reason, Leishman bases his decision as to whether he is dealing with 'old' or 'young' arterioles on the age characteristics of the fundus as a whole. "Old" arterioles are found in a senile appearing fundus, in which the optic disk is pale and slightly hollow, the ocular media are hazy, the retinal reflexes are diminished so that the retina appears dull, there is some disturbance of retinal pigmentation and colloid bodies are present.

"Young" retinal arterioles are found in a youthful appearing fundus, in which the media are clear, the retinal reflex is brilliant, the disk is of a healthy pink color, retinal pigmentation is uniform and no colloid bodies are seen.

Leishman expressed the opinion that it seems to be assumed by most authors that the arterioles are universally capable of diffuse increase in tonus, and that the possibility that arteriosclerosis may play a part in modifying the course of hypertension appears to have been disregarded. The normal aging processes in the walls of arteries are either omitted or dismissed too lightly in most discussions of arteriosclerosis. A few cases of primary essential hypertension, Leishman said, run a fulminating course; the remainder constitute the group of so-called benign essential hypertension. Individual cases in this group will show great variations in course and severity. The difficulty in classifying these cases is due to the failure to appreciate the importance of the arteriosclerotic factor"[10].

Ophthalmoscopic examination of the ocular fundi will aid in the differentiation of the types of vascular symptoms on which the hypertension is implanted since it permits ready recognition of old as opposed to youthful arteries and arterioles regardless of the actual age of the person.

ATHEROSCLEROSIS. Atherosclerosis[6] and senile changes merge into one another, and thus in many instances it becomes difficult or impossible to determine how far a specific degenerative change in a particular tissue is related to atherosclerosis or to senescence. The two processes are, nonetheless clearly distinguishable.

Atherosclerosis is a systemic disease, the chief, but by no means exclusive, primary manifestation of which is to be found in the arterial walls. The characteristic arterial lesion is the atheroma, which begins as a sharply circumscribed accumulation of fat-laden cells clustering together between the internal elastic lamella and the endothelium of the artery wall. The source of these cells is not entirely clear.

In the eye the lipoid infiltrates of nonvascular tissues produce the lipoid arcus of the cornea in which a deposit, chiefly of cholesterol esters, is found in the corneal stroma near the limbus, in Bowman's membrane, and especially in Descemet's membrane. Similar lipoid infiltrates are also to be found in the sclera in regular association with the lipoid arcus. Friedenwald has shown that the choroid also is involved in the lipoid infiltration, which there, too, is diffuse, uniformly distributed, and not related to the normal pattern.

Atheromatous plaques in the intraocular portion of the retinal vessel are, on the other hand, much less frequent, and are much smaller and less extensive than in the neural part of the artery. Friedenwald has demonstrated, however, that typical atheromatous plaques can be found even in those portions of the retinal tree that are truly arteriolar in character, that is, in vessels that have no internal elastic lamella and no continuous muscular coat.

If we accept Allbutt's differentiation of involutionary sclerosis as an aging process in the arteries, independent of, but sometimes associated with, high blood pressure, we may divide the general population of nonhypertensive subjects in two groups in order to consider the effect of high blood pressure upon them: 1) Those significantly affected by involutionary sclerosis. 2) Those not affected.

Involutionary sclerosis is of almost universal occurrence after the sixth decade (Duke-Elder, 1940), and must be, therefore, regarded as a normal concomitant of old age. It may occur

prematurely in younger persons, whereas in some elderly people it may have progressed little at an advanced age. It commonly affects the aorta and larger vessels but may also be found in the arterioles[8].

In cases of senile ocular diseases accompanied by hemorrhages, investigations of the blood coagulation system have shown that besides the vascular changes which are visible by ophthalmoscopy, there are also deficiencies of the blood coagulation system.

Senile macular degeneration is almost always bilateral. Two types may be differentiated:

The first form occurs in old age and shows slight pigmentary changes. Dark red or yellowish dots appear in the dark area in the center of the macula. Small deposits of pigment may be observed. The visual acuity may be disturbed early in the course of the disease. There is no metamorphopsia, however, but in the beginning there may be a central scotoma which is larger for blue than for red. The area of pigment displacement spreads very little. It may become as large as the disc and often somewhat lighter in color due to the disappearance of the pigment epithelium. The fine displacement of pigment may be observed after dilation by means of direct ophthalmoscopy. In advanced cases there is temporal pallor of the disc. The disease progresses very slowly. Such changes have often been called central chorioretinitis and were believed to be due to syphilis. In most cases they are probably caused by sclerotic changes in the choriocapillaries of the macula area and the nutrition of the external retinal layers is seriously disturbed. That is why vasodilators have been recommended to stop the progress of the disease.

The second form of senile macular degeneration is characterized by a lighter colored area, 1 to 2 D.D. in size. It is well defined and caused by atrophy of the pigment epithelium. Such areas are similar in both eyes. Most of them are irregular and have scalloped borders. A disturbance in central vision is present, but is not marked at first. The central scotoma is not always detectable. Senile circumpapillary atrophy of the choroid may also be present, and points to the cause of the disease, namely, a vascular disturbance in the choroid as well as in the retina.

The pallor and loss of pigment in the pigment epithelium does not necessarily indicate that the epithelium is completely destroyed. More often, however, there is a change in the pigment epithelium rather than destruction of it. The cells lose pigment, become flat and irregular, but they still supply some nutrition to the external layers. If one considers the history of such cases it becomes apparent that macular degeneration is rarely familial or hereditary.

In exceptional cases, sharply defined atrophy of the pigment epithelium may not be present in the second group. Instead there is a large white area covered by gray spots. These spots are poorly defined and may reach a size two or three times that of drusen. Deposits of pigment lie between them. The presence of a central scotoma may point to a true circumscribed macular lesion. Since this disease develops very slowly, changes little, and is confined to old people, it is properly classified as a type of senile macular degeneration. The round spots apparently correspond to thickenings of the lamina vitrea. They may be drüsen-like deposits.

Bedell *et al.*[3] reported that senile macular degeneration and Kuhnt-Junius disease are the same. We should consider the two common forms of

senile macular degeneration. The first evidences of one are perimacular flat, deep retinal, or superficial choroidal hemorrhages which enlarge and vary in form with exudates appearing near the center of the extravasation. The exudates and hemorrhages increase until finally, often after years, and after having passed through the circinate phase, the hemorrhages cease and the exudate becomes an organized scar. In the other type of senile macular degeneration, vessel sclerosis and macular edema and hemorrhages are less conspicuous but often recur until the terminal stage when there is an almost flat macular region with gross choroidal vessel sclerosis.

Dr. Kravitz said there is no depreciation in vision of the aged with demonstrable changes in the media or macula. Dr. Kornzweig quoted from an article by Fisher that 50% of old people have decreased vision without findings. Elwyn said that he has seen diminution of vision with no pathologic process evident in media or fundi. Waardenburg reported two sets of aged female monozygotic twins who both suffered from senile macular dystrophy[9].

Separation of the layers of the retina "retinoschisis," occurs usually in the temporal fundus of persons in the 50- to 60-year bracket and males are more frequently affected than females. The lesions are of comparable extent in both eyes. The inner layer of the retinoschisis always contains the retinal blood vessels, and they often appear white in their periphery as if obliterated.

At first it appears to be nothing more than an exaggeration of cystoid degeneration of the peripheral retina; however, in its second stage, senile retinoschisis progresses in two directions, it gradually extends nasally until it finally encompasses all of the extreme fundus periphery; it progresses from the temporal region toward the disc. In its third stage it forms a large cyst-like elevation in the inferotemporal or supero-temporal quadrant.

There are several conditions with which retinoschisis may be confused, for instance, retinal cyst, retinal detachment, choroidal melanoma, choroidal exudative disease. As retinoschisis disrupts the layers of the retina, it is futile to attempt restoration of visual field by reattaching its inner layer. Surgery rarely succeeds in permanently flattening out a retinoschisis.

REFERENCES

1. **Ashton, N.**: Glaucoma. Tr. 4th Conference, Josiah Macy, Jr. Foundation, p. 127, 1959.
2. **Becker, B.**: Am. J. Ophthal., **46**, 731, 1958.
3. **Bedell, A. J., Elwyn, H., Kravitz, D.,** and **McLean, J. M.**: Ibid., **38**, 235, 1954.
4. Elwyn, **H.**: Diseases of the Retina, 2nd ed. New York and Toronto: Blakiston Co., Inc., 1953.
5. **Friedenwald, J. S.** *et al.*: Ophthalmic Pathology. Philadelphia: W. B. Saunders Co., p. 45, 1952.
6. Iidem: Ibid., pp. 312-314.
7. **Kornzweig, A. L., Feldstein, M.,** and **Schneider, J.**: Am. J. Ophthal., **44**, 31, 1957.
8. **Leishman, R.**: Brit. J. Ophthal., **41**, 643, 1957.
9. **Waardenburg, P. J.**: XVIII International Congress of Ophthalmology. Excerpta med., C27, 1958.
10. **Wagener, H. P.**: Am. J. Med. Sci., **241**, 249, 1961.

SURGERY

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TRANSPLANTATION OF TISSUES

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During the past 20 years intense interest in the transplantation of tissues has been kindled anew, and a number of excellent reviews of various aspects of the field have been published[11,23,34,44,54]. But while a better definition of the problem has been developed, the biologic barrier to successful homotransplantation between nonrelated hosts has yet to be surmounted. Essentially, the problem is as follows[34]: if skin from a man's arm be transplanted to a suitably prepared place on his leg, it develops a blood supply and lymphatic drainage, and it becomes more or less indistinguishable from surrounding tissues. If this same skin were to be placed on the leg of a nonrelated person, however, the sequelae would be quite different. During the first 5 or 6 days this "homograft" would be indistinguishable from an "autograft." It would become vascularized, acquire lymphatic drainage, and appear to have survived. Yet, within 7 to 18 days it would become edematous, inflamed, and would eventually ulcerate. This is the "first set reaction." If a second piece of skin from the original donor were now to be grafted, the second graft would be rejected several days sooner than the first. This accelerated rejection is termed the "second set reaction."

This example of transplantation of skin is applicable to the transplantation of most nucleated tissues with very few exceptions. The accelerated rejection of the second graft suggests that the host has developed "immunity" as a result of exposure to antigens from the donor. Exploration of this immunity has been the objective of an immense amount of research[18]. The extensive work of Medawar and associates[7,8,30-32] between 1940 and 1960 categorized intolerance to transplanted tissue as an actively acquired immunity, and today this is the most universally accepted concept[5].

Solution of the "homotransplantation problem" would provide a tool of tremendous clinical potential. Whole organ replacement between nonrelated persons would then become possible, and surgical procedures for transplantation, already having been perfected, would immediately be carried out. As a side product of homotransplantation research, a body of new immunological concepts would be defined which might well be applicable in the treatment of malignancies and perhaps the autoimmune diseases. The body's barrier to transplantation of tissues between nonrelated individuals having been broken, homotransplantation would then be as feasible as the transplantation of tissues between identical twins[73] is now.

DEFINITIONS. Before discussing homologous transplantation of tissue, to which the subsequent discussion will be largely confined, certain terms should be defined:

1. *Chimera:* the name given to an animal harboring the tissues of an animal of different genetic constitution.
2. *Heterograft:* tissue transplanted from donor to recipient of a different species.
3. *Homograft:* a transplant from donor to recipient of the same species.
4. *Autograft:* a graft from an individual to the confines of that same individual.
5. *Isograft:* a graft from one uniovular twin to the other twin.
6. *Homostatic Graft:* a graft whose presence as a nonviable tissue may serve a useful purpose in bridging or filling a defect (arterial graft).
7. *Homovital Graft:* tissue grafts which depend for their success upon their surviving as living functioning tissue.

Homograft Rejection Phenomena. In his investigations of transplantation immunity, Medawar[30] demonstrated that homograft sensitivity was not confined to the area of the first set reaction but was systemic. This was accomplished by taking several pieces of skin from the original sensitizing donor and placing them at various locations on the host. He noted that they regressed simultaneously and postulated that the rapidity of rejection varied directly with the degree of genetic difference between the donor and the host. It was predicted that success in abolishing homograft sensitivity would be furthered by a precise definition of the antigens and antibodies which mediate this sensitivity[26].

There are two phases of homograft rejection[43]: 1) an early, nonspecific reaction characterized by local cellular infiltration at the site of the graft, and 2) an antigen-antibody reaction which occurs after the grafted material has served as an antigen and the host has had sufficient time to respond by forming specific antibodies. Basically, homograft rejection is thought to be a function of the lymphoid and reticuloendothelial systems, and antibodies produced by the host are believed to exist as both humoral and cell-bound types. The fact that homografts survive longer in patients with congenital agammaglobulinemia offers some proof of the importance of humoral antibodies. However, the persistence of viable homografts in the millipore diffusion chambers which exclude contact of sensitized host lymphocytes with the homograft attest the importance of "cell-bound" antibodies. Lawrence[26,27] postulates four types of immunological effector mechanisms, any one or a combination of which could be set in motion by antigenic stimulus and result in the destruction of the homograft tissue concerned: 1) the homograft rejection could be mediated by serum antibodies of the classical type with the

mechanism of tissue damage being analogous to the Arthus sensitivity; 2) mediation of rejection could be effected by a cell-bound immune factor or factors, and the mechanism of tissue destruction would be analogous to tuberculin sensitivity; 3) a combination of 1 and 2; and 4) mediation could be by an immune response which is unprecedented in immunology and which is only now being characterized[27].

Those who believe that the immunological mechanism is analogous to tuberculin sensitivity give the following reasons: 1) the responsiveness of the host is relatively rapid, long-lasting, and systemic; 2) the inflammatory process is characterized histologically by round-cell infiltration and necrosis; and 3) transfer of sensitivity may be accomplished with viable cells[9].

The work of Algire[1-3] with the millipore chamber and similar work by others[10] have demonstrated the importance of cell-bound antibodies in homograft rejection mechanisms. The role, if any, of circulating or humoral antibodies is the subject of much speculation[9,27,34,46]. Most investigators are of the opinion that humoral antibodies play a minor part in the homograft rejection phenomenon. Four types of humoral antibody activities have been recorded in immune sera[49]: a) erythrocyte agglutination, b) leukocyte agglutination, c) protective tumor neutralizing effect, and d) cytotoxic effect. Whether multiple or single antibodies are responsible for these phenomena remains to be seen. It is generally believed that the process of active immunization entails the formation of antibody-like reactive sites either on the surface of cells of the regional lymphoidal tissue or within them, and it is these reactive sites which play the dominant role in the destruction of the transplant[6].

Consideration must now be given to the antigens which presumably elicit homograft sensitivity. It has been found by testing the antigenicity of cell debris separated by differential centrifugation that most of a cell's antigenicity resides in the nuclear tissue and little or none in the cytoplasm. This is borne out by the fact that non-nucleated cells are unable to elicit immunity in adults or tolerance in young animals. They are under strict genetic control and thus successful transplantation requires that all the dominant genes be present in donor and host, a condition which exists in identical twins and highly inbred strains of animals. The number of antigens varies with the species. Medawar[30] postulated 5 in the rabbit, but as many as 27 have been postulated in man. He and others[31] believe that the tissue antigens are complexes containing deoxyribonucleic acid, but the use of DNA alone has not yet produced homotransplantation immunity. The genetic laws applicable to transplantation antigens are as follows[17]:

1. Transplants within a single inbred strain (A→A) grow progressively in all hosts.
2. Transplants between strains (A→B) do not grow, or grow temporarily and then regress.
3. Transplants to F_1 hybrid animals where one parent was inbred and susceptible (A→AB) grow progressively in all hosts.
4. Transplants originating in F_1 hybrids will grow in F_1 hybrids (AB→AB) but not in members of either parent strain (AB→B; AB→A).

These are known as Snell's laws.

Vascularization of the homograft is important. Converse[12] believes that the degree of hypersensitivity developed by the host is directly proportional to the amount of vascularization of the graft that has occurred. It has been noted many times that as long as grafts to

the anterior chamber of the eye remain avascular, they will remain viable. It is postulated that the antigens reach the host *via* the graft's lymphatic drainage system and that the antibodies reach the graft *via* the blood vessels. The brain with its poor lymphatic drainage is a suitable place for prolonged homograft survival[27]. Whereas the cell nucleus serves as antigen, cell cytoplasm is considered to be a site of antibody formation.

To recapitulate, both a "primary response" and a "secondary response" are manifest in homograft reaction phenomena. In the primary response an initial "take" period is noted which lasts no less than 5 or 6 days. During this time it is indistinguishable from an autograft, and it develops a blood supply and lymphatic drainage. This first 5 or 6 days is also a latent period during which the host's response is set in motion. After the fifth day a round cell invasion of the graft is noted. These are chiefly immature lymphocytes and plasma cells. When dilatation of the graft vasculature occurs, rejection is impending. With this dilatation there follows vascular congestion, and circulation in the graft ceases. The endothelium breaks down, and widespread hemorrhage occurs. The graft epithelium degenerates, causing fragmentation, erosion, and destruction. The graft is finally undermined by the host's skin and is sloughed[9]. The "secondary response" or second set reaction occurs when the host has been previously sensitized to the donor tissue, and the second graft is destroyed more rapidly. This immunity is present over the whole body surface and the interior, representing acquired immunity. In the rat this immunity has been shown to extend at least over a 240-day period. The histological appearance of the second set reaction is different from the primary reaction. No vascularization occurs and no cellular infiltration develops at the graft interface. The primary reaction is thought to leave the host tissues in a general state of sensitization in much the same way as tuberculin sensitivity.

Homograft Takes: Measures Used to Enhance. Various methods have been used to prolong the life of homografts. The measures employed have been designed to alter the donor, the specific tissue to be grafted or the recipient. For example, the host has often been irradiated prior to excision of the tissue to be homografted. Treatment of the graft might include irradiation or lyophilization. Some effort has been made to reduce, if not abolish, graft antigenicity with tissue culture techniques. In cases where the graft is an organ such as intestine, heart, liver or a limb, perfusion and simultaneous cooling to temperatures in the range of 4° C. have been carried out using Ringer's lactate[5,25].

Treatment of the host has also been the focus of intense effort. Generally, the modalities of treatment are directed toward depression of the immunological response. These have included irradiation, the use of endocrine substances such as cortisone, chemicals including benzol, nitrogen mustard and other cytotoxic agents[33,43], and reticuloendothelial blockade with particulate matter. All of the above mentioned methods of treatment have met with only partial success.

ACQUIRED TOLERANCE. A further concept has been evolved which is potentially useful. This is the concept of acquired tolerance. Acquired tolerance is a state of tolerance for homotransplants which is induced by the introduction of cells from the proposed donor into the proposed host while the host is in the embryological or, at the latest, the neonatal state. It has been demonstrated many times that after

this early treatment the host later, when adult, will accept further grafts from the original donor.

The historical background for this concept came from observations that fraternal cattle twins which are synchorial will accept each others' skin grafts. It was proposed that in the embryological state these animals had a common placenta which permitted mixing of blood elements. It is further noted that grafts from mothers to their offspring show a greater incidence of prolonged survival than grafts from fathers to their progeny. This is considered to represent more evidence that acquired immunity due to the mixing of blood elements may develop due to transplacental exchange. In such situations the individuals may become chimeras in that each may harbor cells from the other. Incidentally, leukocytes from a female animal can be identified following their injection into the blood stream of a male through differences in cell morphology.

In an effort to explain the phenomenon of acquired tolerance, Billingham, Brent and Medawar[7,8,30-32] evolved the following theory: they proposed that tolerance could be produced by the introduction of foreign tissue into the embryo before it reached a critical age at which its immunological mechanism could differentiate "self" from "not self." Before this critical age, the foreign tissue would be taken in and considered "self" when the critical age is finally reached. Subsequently, further grafts from the same donor would produce no homograft reaction. This is a form of actively acquired tolerance and so far it has been demonstrated to be permanent. The period of the embryo's existence before it can distinguish between self and not self is appropriately termed the "adaptive period." It has been found that the earlier the introduction of foreign cells, the easier it is for the embryo to adapt[34]. Acquired tolerance has been applied to heterologous tissue also. Any nucleated tissue from the donor is sufficient to produce acquired tolerance. Following the "adaptive period" there is a "null period" during which the embryo is able to distinguish foreign tissue but is immunologically unable to react. Homografts are 90% rejected during this period. Following the "null period" there is the period of "immunological maturity."

An ingenious method for the introduction of foreign tissue is one called *parabiosis* in which two animals' circulations are connected. The phenomenon of acquired tolerance may be demonstrated[20] using this method, one which has been employed in biologic study for almost a century. It has also been found that acquired tolerance can be transferred to another animal *via* parabiosis.

Concurrent with the findings mentioned above concerning acquired tolerance, it was noted by Toolan[50] and others[18,47] that fetal tissue, especially skin of embryos, was less antigenic up to a certain age, and thus that embryonic skin grafts survived for a longer period when placed on adult hosts.

GRAFT VERSUS HOST (RUNT) DISEASE. An unfortunate result of the production of acquired tolerance is the production of secondary disease. This entity is termed "runt disease," "graft versus host disease," or "parabiotic disease." This entity may result when chimerism is produced by embryonic tolerance, by parabiosis, or by the use of irradiation to destroy the host's immune mechanism. Graft versus host disease is characterized by failure of growth and development of the host, diarrhea, anemia, spherocytosis, an elevated serum indirect bilirubin level, lymphatic atrophy, and pulmonary edema. This reaction pattern has been

noted by many workers[34,38,41,42,51].

The immunological explanation for this adverse phenomenon is that it results from antigens being released by the host, and that the immunologically active graft produces antibodies which destroy the immunologically inactive host. Proof for this hypothesis lies in the fact that the host demonstrates no hematopoietic depression even though CR_{51}-labeled erythrocytes disappear from the host's blood at a rapid rate. It is also notable that this disease is not evident when isologous tissues are used. The cause of death may be an immunologic hemolysis.

An interesting sidelight in consideration of the transplantation problem is the finding that some cases of chronic illness, such as uremia and thermal burns, have been associated with increased homograft tolerance. Agammaglobulinemia victims, especially those with the congenital form, are tolerant[45]. The same is true of patients with Hodgkin's disease where the reaction to skin homografts is delayed, prolonged, and incomplete in many instances[24]. This is ascribed to a high incidence of anergy in such individuals.

Organ Transplantation. The general concepts of homograft tolerance and rejection have been outlined. In these observations the reaction to skin has been used as a prototype. The remainder of the discussion will be devoted to a brief survey of experiences with the transplantation of whole organs. Transplanted organs are not permanently tolerated by nonrelated hosts, but much has been learned experimentally. The fact that kidney transplants between identical twins have been successful has served as a strong stimulus to further assault upon the immunologic barriers which prevent successful permanent organ takes in nonrelated individuals. Representative organ transplants reviewed will include the heart, lung, liver, bowel, kidney, parathyroid and blood (bone marrow). Arterial homografts have of course been extensively and successfully used as a temporary bridge into which and across which host tissue may grow, but discussion of arterial grafts is not particularly useful in characterizing the homograft rejection phenomena.

HEART. While successful temporary transplants of the heart had been achieved long ago in animals by placing the heart in the neck or elsewhere[29], one of the first complete homologous heart transplants which functioned as the sole support of circulation in the animal would appear to have been that of Neptune and associates[39]. These workers reported 3 dogs in which the heart and lungs were successfully transplanted under hypothermia, with the donor heart maintaining all circulation and allowing return of reflexes, spontaneous respiration, restoration of normal body temperature and survival up to 6 hours following surgery. Electrocardiograms performed on these animals revealed an essentially normal tracing in some instances. They pointed out that the experimental procedure provided a means of studying the denervated heart and lungs, though of course the survival of their animals was of limited duration. As noted, a number of workers had previously placed the heart in the neck and reestablished circulation through it by appropriate anastomoses to the carotid artery and jugular vein. The conspicuous advantage of this location of the heart is that by its pulsations one can identify the functional integrity of the transplanted tissue, which continues beating until the immunologic mechanisms responsible for homograft rejection have become effective to the extent that cardiac contractions cease.

Webb and his associates[53] in our laboratory reported a considerable experi-

ence with the transplantation of heart-lung complexes. After confirming the fact that the heart might survive without circulation for periods up to 90 minutes at room temperature, if as suggested by Crowell and Smith[14] it were to be heavily heparinized or if the blood were removed from the cardiac capillary bed to prevent the formation of obstructing clots within the coronary circulation, they added the additional protection of refrigeration in a nutrient medium at 4° C. This offered satisfactory preservation for at least 6 to 8 hours with return of normal function. Of 12 technically successful transplants utilizing refrigerated hearts, 10 were able to maintain normal blood pressures for from 30 minutes to 7½ hours. For example, an animal would recover and be capable of walking about the laboratory while his circulation was maintained entirely by the transplanted heart.

Such experiments clearly emphasize the fact that, with additional physiologic and technical exploration, the transplantation of hearts would appear to be feasible if and when the immunologic problems incident to homotransplantation have been overcome.

LUNG. Lung transplants have been mentioned in connection with heart-lung transplantation, but the transplantation of the lung itself is revealing because it imposes less stress upon the recipient and thus permits longer survival and more precise functional analysis. Although various workers had removed a lung and then reimplanted the same lung at its original site successfully with apparent satisfactory function in this organ, Hardin and Kittle[19], and Neptune, Weller and Bailey[40] were among the early workers who successfully transplanted lungs from one unrelated animal to another. After working out various technical problems utilizing the autogenous re-implantation technique, Neptune *et al.* performed 25 homologous lung transplantations. In general, the clinical course of the animals followed a consistent pattern. They appeared to be in excellent condition initially and on the day following surgery they would eat and walk willingly on a leash. There was good ventilation on the side of the homologous transplant, as evidenced by auscultation and Roentgen examination of the lungs; bronchoscopy also revealed a patent bronchus and adequate ventilation. After several days, however, the animals became listless and refused to eat, dying thereafter. At necropsy the transplanted lung was grossly enlarged in size and weight and was dark red and appeared solid on palpation. On sectioning, widespread necrosis was noted and no gross pattern consistent with healthy pulmonary tissue was found. It was the impression that the administration of ACTH had permitted certain lungs to survive longer than those in animals not so treated. The average survival time without the use of ACTH was 6 days; with ACTH it was 25 days.

Hardin and Kittle[19] carried the technique of lung transplantation further in that following transplantation of a lung to one side of the animal the opposite lung was also removed in 5 dogs. This required the entire respiratory support of the animal to be maintained by the transplanted homologous lung. In 3 animals death occurred either during the operation or within 20 minutes after its completion. However, the remaining 2 animals survived for 6 and 9 days, proving unequivocally the functional ability of the homologous lung during these periods.

Here again, as with heart transplantation, the fact that the animals can survive for the duration of the usual period of tolerance of homografts and the graft provides adequate pulmonary

function in the absence of the animal's excised lungs, is proof that it is not technical difficulties but the immunologic barriers which thus far prevent the successful use of lung homotransplantation in the treatment of pulmonary insufficiency due to various diseases such as emphysema, cystic disease, or tuberculosis.

LIVER. Certain organs have been far more extensively investigated as homotransplants than have liver transplants: skin is easily available; the kidney has significance as a paired organ with a simple vascular pedicle and a biochemical product which can easily be measured; blood is needed for circulatory support; and bone marrow is useful as a replacement tissue after hematopoietic destruction either in cancer treatment or as a preliminary to other transplants. Nevertheless, few of these organs are the seat of a metabolic activity so extensive as that of the liver. Accordingly, Moore and his associates[36], as well as other workers, have investigated the techniques and physiologic function of homologous liver transplants in animals. In beginning their studies, they also noted the well established principle that at present clinical homotransplantation must rest on a state of immune tolerance achieved by chimerism, in which donor cells reside in the host without exciting an effective immune response. Should it be possible to attain destruction of the immune system, as with irradiation or cytotoxic drugs, followed by hematopoietic restoration as with infusions of bone marrow or spleen, the recipient might then accept and tolerate other tissues from the same donor whose cells were responsible for the hematopoietic restoration. Drawing upon previously reported techniques for transplanting liver tissue in animals whose own liver was left *in situ*, Moore and his group worked out a satisfactory operative routine. It was found that the short-term hepatectomy suffered by the recipient dog was not a serious problem. The transient anoxia to which the transplanted liver was subjected would have been a major problem had it not been for the remarkably beneficial effect of cooling upon the maintenance of hepatic cellular integrity during anoxia. One major problem in liver removal is that the anticipated recipient animal has no vena cava at that level temporarily and this results in pooling of blood in the kidneys, mesenteric area, and lower extremities. Yet, if simultaneous aortocaval occlusion is instituted for a protracted period, a severe acidosis of the distal portion of the dog is produced, which will result, upon reopening of the occlusive clamps, in a severe hyperkalemic hypoxic acidosis, cardiac arrhythmia, shock and death. This problem was solved by the use of two low-pressure shunt systems from lower caval and portal systems, respectively, to the jugular veins. The homotransplant procedure was undertaken in 31 animals, of which there were 15 survivors for more than 24 hours. Eight animals lived more than 4 days, 2 more than 5 days, and one each survived 5½, 6, 8 and 12 days.

In the animals surviving the first 24 hours there was a characteristic clinical course. The animals regained consciousness and became able to walk about. Although they recognized their handlers and produced an adequate volume of urine, they were unable to ingest significant amounts of food. Somewhat surprisingly, death was often unassociated with liver failure and was usually unaccompanied by hypotension or shock except terminally. The terminus did not appear to these workers to represent a fatality from hepatectomy *per se* in most of the animals, but was due to one or more of a variety of clinical

complications. In addition to transplantations of the liver, transplants of spleen were also carried out. Controlled experiments consisted of autotransplantation of the liver and spleen, returning these organs to their normal sites. In the case of both the liver and the spleen a satisfactory surgical technique was achieved. Rejection of these homotransplanted viscera was a gradual cellular process, sharply differentiated from avascular necrosis or surgical failure. It was pointed out that liver replacement could be useful in cirrhosis and in replacement of the liver where this organ was the only site of tumor, should immunologic barriers be overcome.

Of particular interest were chemical studies of the blood following liver transplantation. The blood urea nitrogen level was not significantly elevated in most animals. The blood sugar level was usually normal or elevated, but when elevated there was often the possibility that intravenous glucose infusion might have been a causative factor. The alkaline phosphatase level exhibited a progressive increase which often antedated a rise in the bilirubin concentration. The serum bilirubin concentration was usually normal or slightly elevated during the first few days following operation, but rose progressively prior to death. The plasma albumin and globulin concentrations were studied by paper electrophoresis, and a fall in albumin and a rise in alpha-2 globulin were characteristic. As for blood coagulation studies, no significant abnormalities were noted in these factors until at or near the time of death when a bleeding tendency associated with a low prothrombin level became clinically evident.

BOWEL. The development of effective techniques for small vessel anastomosis has permitted an approach to transplantation of the small bowel. Among studies of this problem, the work of Lillihei, Goott and Miller[28] has been outstanding. Drawing upon the techniques available for the suture of small vessels and especially veins, as well as the beneficial effect of cooling the bowel to be transplanted where necessary, these workers reported the physiologic response of the small bowel to ischemia, including prolonged *in vitro* preservation of the bowel with successful replacement and survival. In addition to extensive studies with replacement of the bowel into the same animal, successful homotransplantations of the small bowel were also carried out. However, inasmuch as the discussion of their homograft experience was brief, the experiences of Barnett[4] in our laboratory may be cited. He and his assistants performed 20 homografts of small bowel using the following technique. A segment of bowel one foot in length was selected and the dissection was taken deep into the mesentery to provide a relatively large artery and vein. The continuity of the remaining bowel was then reconstituted by anastomosis. The artery and vein of the excised small bowel segment were sutured to an iliac artery and vein respectively. One or both ends of the transplanted bowel were brought out through the abdominal wall, and subsequently various substances, including the deuterium oxide and radiosodium, were employed to determine rates of absorption in the transplanted loop daily. The progress of the rejection of the homografts was readily apparent on gross inspection and on cutting of the exteriorized ends of the bowel loop. The exteriorized portions of the transplant were seen to become progressively darker and more cyanotic and cutting of the bowel resulted in less and less bleeding as the days passed. The longest survival of a homograft

was 9 days. Rejection, necrosis and absorption of the bowel transplant resulted in death of the animal in all instances except one. In this dog the bowel had reached the stage of a fibrous cord and had been walled off by loops of bowel within the abdomen.

It is clear that rejection of bowel homografts follows approximately the same biologic process as does rejection of heart, lung and liver as described previously. Nevertheless, here again it has been demonstrated that an important organ can be technically transplanted successfully, only to see the transplant eventually rejected because of the immunologic barriers to transplantation between genetically different partners.

KIDNEY. One of the most dramatic and successful results of isologous organ transplantation has been the transplantation of one kidney of one identical twin to the other twin[37], as advanced by the group at the Peter Bent Brigham Hospital. These successful transplants between identical twins followed earlier kidney transplantations between nonrelated persons[22]. In the original studies, at which time the technique of kidney transplantation was further developed, a kidney was transplanted from a cadaver to a patient with severe renal damage in the forty-fifth day of anuria. This patient died 37 days after the transplantation and the renal transplant was found to have developed a severe glomerulonephritis similar to that seen in the patient's own kidney. This suggested that under certain circumstances the transplant was affected by the disease of the host, and that perhaps the patient's own kidneys should be excised following insertion of a transplant even from an identical twin.

Murray, Merrill and Harrison[37] reported kidney transplantation between 7 pairs of identical twins in 1958, and a number of others have since been added to this series. The indications for transplantations between identical twins were as follows: The healthy potential donor must have evidence of normal renal function bilaterally, be free from infection, have a normal lower urinary tract, and have sufficient understanding of the significance of the unilateral nephrectomy. As for the recipient, he or she must be in a terminal state of irreversible uremia and have a normally functioning lower urinary tract. A malfunctioning lower urinary tract with poor or incomplete emptying might ultimately impair a transplanted kidney by pressure or subsequent infection. Infection, if present, should be minimal or controllable, and the primary renal disease should be inactive. Murray and his associates have felt that genetic and immunologic identity between host and recipient should be proved in all potential candidates for transplantation by cross skin grafting as well as the examination of blood types for all known blood groups. Final proof was considered to be the presence of a nonreactive skin graft observed grossly and stereomicroscopically on the healthy donor after 3 weeks. In transplants between minors under 21 years of age, a declaratory decree from the Supreme Court of Massachusetts was considered necessary to permit the operation in the healthy twin because, in such situations, it was felt that neither the parents nor the healthy child had the right to consent to an operation which was not for the donor's benefit.

The operative technique employed by most surgeons performing human kidney transplantation is basically as follows. The nephrectomy on the healthy donor is carried out in one operating room. In another room a position for the donor kidney is prepared in the right iliac fossa of the recipient: A right lower quadrant retroperitoneal

exposure is made to identify the iliac vessels from the bifurcation of the aorta to the inguinal ligament. All the terminal branches of the hypogastric artery are dissected out, care being taken to preserve each branch for possible use if renal abnormalities are encountered in the donor. The retroperitoneal area is dissected widely in a lateral direction to allow room for the kidney. In general the renal artery is anastomosed end-to-end to the hypogastric artery and the renal vein end-to-side to the external iliac vein. If two or three renal arteries are present, as not infrequently is the case, suitable branches of the hypogastric are selected. As for the ureter, this structure is passed through a muscular tunnel and sutured to the bladder mucosa.

Murray, Merrill and Harrison[37] found that the time lapse from clamping of blood supply of the donor kidney to its restoration in the recipient ranged from 55 to 75 minutes in the 7 cases referred to. One conspicuous finding was the fact that urine flow began immediately in most cases, and by the conclusion of the operation a copius diuresis was in progress. A ureteral catheter to the renal pelvis of the transplant was used occasionally. The postoperative function of these kidneys was excellent. A striking feature in the recipients was the rapid restitution of well-being and appetite. Convulsions ceased, congestive heart failure disappeared, and the blood urea nitrogen level declined. Following an early output of a large volume of dilute urine, renal function progressed rapidly towards normal during the subsequent months. Patients with severe hypertension exhibited a decline in blood pressure but not quite normal, with the transplantation of a third (normally functioning) kidney. However, pressure returned to normal when the recipient's own two kidneys had been removed.

Despite the impetus and encouragement derived from these successful transplants of kidneys between identical twins, such isologous transplantations do, of course, beg the question of homograft rejection. Nevertheless, following these studies continued efforts were made to make a successful renal transplant in nonidentical twins, and a successful homotransplantation of the kidney between nonidentical twins has in fact been reported by Merrill and his associates[35]. A kidney was successfully transplanted between a nonidentical twin and his brother after whole-body radiation of the recipient but without infusion of bone marrow. The skin transplanted from donor to recipient was rejected 8 months after grafting, emphasizing the presence of close genetic relationship but the absence of complete genetic identity.

Hume and his associates[21] have presented a detailed study of the homotransplantation of kidneys and of fetal liver and spleen following total body irradiation. They utilized the knowledge that if a sufficiently large dose of total body irradiation be administered to mice, antibody formation is suppressed but marrow transplants will take and persist. The repopulation of the hematopoietic system by the grafted marrow leads to the production of an antigenic mosaic pattern characteristic of the donor rather than the recipient. Radiated mice with bone marrow homotransplants will therefore accept skin homografts from members of the donor's strain. However, the use of bone marrow homotransplants after radiation may lead to the appearance of a significant number of animals with "runt syndrome," in which failure to grow and eventual death appear to be related to the reaction of the graft against the host as described previously in this discussion. Yet, if fetal hematopoietic tissues, particularly liver and

spleen, are used instead of adult marrow, the "runt syndrome" may not appear. It is believed that this is because the fetal tissue, by virtue of its immunologic immaturity, is able to acquire a tolerance to the host's antigens and thus does not react against the host[21,53].

It has been noted that rejection of homografted kidneys in the dog is accompanied by the appearance of lymphocytes and plasma cells in the interstitial spaces of the cortex, accompanied by edema, tubular destruction, and cessation of urine formation. It was suggested by Dempster[15] that the round cells which appear in the interstitial spaces are of donor origin and may represent a reaction of the donor against the host. Thus Hume and his associates[21] attempted to determine 1) whether infusions of homologous fetal liver and spleen cells would permit indefinite survival after lethal doses of total body irradiation in dog, 2) whether irradiation of the host prior to renal homotransplantation would prevent the appearance of the usual homograft rejection phenomena, and 3) whether irradiation of the donor prior to transplantation would eliminate the homograft rejection phenomena. Twenty-eight dogs were given 600 r total body irradiation and all died within 7 to 17 days. Sixteen dogs were given 600 r total body irradiation plus infusions of fetal liver and spleen cells. Four of this group were surviving 12, 16, 18 and 22 months later. In one of the dogs in which a male host was given female embryonic blood forming cells, female leukocyte markers could be readily detected in the peripheral blood of the host up to 8 months, but had disappeared by 12 months after irradiation. No long-term survival was achieved with the use of fetal tissues in a few cases in which total body irradiation dosage of 1,000 r or greater was used. Homotransplants were not facilitated by irradiation of donors with dosage up to 1500 r; the homotransplants were rejected in the usual way in the usual period of time. Likewise, irradiation of the host at doses of 600 or 1,000 r failed to prevent rejection. Irradiation of the host in doses of 1200 r prevented rejection in half the cases, and irradiation at doses of 1500 r prevented rejection in all cases. Irradiation of both host and donor at 1,000 r or greater prevented rejection in all cases, but not at doses of 600 r. Long term survival of renal homografts was not sought in most cases, the study being directed primarily at the appearance of functional and histological evidence of rejection 3 to 13 days after transplantation.

These studies in renal homotransplantation, while not definitive with respect to the successful achievement of permanent renal homotransplants, have been cited in some detail because they emphasize the general direction which efforts to improve successful renal homotransplantation between nonrelated donors have taken. In general, irradiation or radiomimetic drugs have been the chief agents used to diminish the antigenic potential of the transplanted tissue and to reduce the immunologic response of the host. Although a variety of drugs have been employed, and in particular 6-mercaptopurine, these have not been conspicuously more successful than the irradiation results outlined above.

PARATHYROIDS. Among the earliest efforts at transplantation were those involving endocrine glands. Actually, implants have been employed far more often than transplants, the former being implanted as slices without effort to anastomose vessels, while the latter represent formal anastomosis of blood supply of the donor organ to vessels in the host. In general, it has been found that endocrine organs appear to

be rejected in much the same manner as are other organs, although there exist rather sharp species differences with respect to endocrine tissue. Perhaps the most remarkable endocrine organ, from the point of view of transplantation, is the parathyroid gland. Jacob, Gowing and Dunphy[23] have reviewed the experience in the transplantation of this and other organs. Of all endocrine glands transplanted, the incidence of success and failure with respect to parathyroid gland transplants and implant has been the most difficult to determine. There have been sporadic reports for many years to the effect that in patients with hypoparathyroidism—whether congenital, spontaneous or postsurgical—implanted parathyroid tissue or transplanted whole glands have ameliorated the hypocalcemia and other symptoms incident to the usual state in hypoparathyroidism. Studies using the millipore chamber[10], which prevents contact of host cells with the donor tissue in the chamber, are intriguing and promising but not yet definitive.

Unfortunately, there are several problems which beset the investigator performing parathyroid homotransplants. First, the state of hypocalcemia may become less severe spontaneously as the months go by, and certainly it may become better tolerated by the patient or animal under study. Second, the parathyroid glands do appear to have a greater resistance to rejection by the nonrelated host than do other tissues. For these reasons it is extremely difficult to be certain that a clinical response has actually been due to a parathyroid transplant with appropriate vascular anastomoses in the given patient, unless one can provide histologic demonstration of apparently functioning and healthy parathyroid cells months after transplantation. In discussing Sterling's report[48] of 3 successful cases, Conway[13] voiced considerable skepticism. He suggested four criteria which should be met prior to the acceptance of a parathyroid transplant as being effective in ameliorating a state of hypoparathyroidism: first, by appropriate balance studies a deficiency in calcium metabolism should be demonstrated before homografting; second, a satisfactory equilibrium of metabolism of calcium in the absence of oral or parental replacement therapy should be demonstrable after homografting; third, a biopsy should be performed to demonstrate functioning parathyroid tissue in the transplant; and, fourth, though rarely possible of fulfillment, one might excise the endocrine transplant and produce a return of the preoperative deficiency state.

More recently Dunphy and Jacob[16] have reported clinical experiences with heterologous (goat) parathyroid implants, but careful study disclosed failure of normal parathyroid cells to survive.

BONE MARROW. It was pointed out above that transplantation of homologous and even heterologous bone marrow may be successfully accomplished in irradiated mice, but this is less successful in larger mammals such as the dog and the monkey. Furthermore, the infusion of homologous bone marrow in dogs is likely to produce the "runt syndrome" in a number of the recipients. It was seen above that the use of fetal hematopoietic tissue is less likely to produce the "runt syndrome" than is that from adult dogs. Whereas the functional integrity of other homotransplanted tissues can usually be followed by either the function of the organ concerned or of inspection and biopsy in the case of the skin, the identification of the transplanted bone marrow is more difficult. However, by making use of the female leukocyte markers, the introduction of bone mar-

row from a female donor into the blood stream of a male host permits the observer to follow the survival of the injected female cells in the peripheral blood. Thus far the long-term or permanent survival of marrow homotransplants has not been successful in the larger mammals, to our knowledge.

Of special interest is the reinfusion of autologous bone marrow. In patients who are to be heavily irradiated as treatment for malignant neoplastic disease or in preparation for homologous renal transplants, it has been a common practice to withdraw as much marrow as possible from multiple bone sources prior to total body irradiation and then to reinfuse this marrow shortly after irradiation. Although there has been some controversy regarding the genuine efficacy of this procedure in protecting the patient against pancytopenia in the days following irradiation, it would appear to be a sound concept, and further experience and improvement in methods and technique may well establish this type of marrow transplant as a feasible and practical procedure. Here again, however, the genetic problem involved in homograft rejection is merely avoided, not solved.

Summary and Conclusions. The nature of the reaction which results in the rejection of homografts has been reviewed. It is widely accepted that this process reflects the development of actively acquired immunity on the part of the host to the tissue from the genetically unrelated donor. The resulting systemic immunity is not unlike that which characterizes the reaction to tuberculin following a previous tuberculosis infection. The nucleus harbors the antigen in large part, but antibodies are formed in cytoplasm.

Transplantation of tissue is successful between genetically identical partners, as with identical or isologous twins, or between highly inbred strains of animals. The presence of agammaglobulinemia in the recipient, resulting in a highly defective immune response, permits longer survival of tissue transplants from nonrelated donors. Embryos and certain neonates, to which homografts are introduced before development of the immunological mechanism, will tolerate these homografts, and later in life they will tolerate additional homografts from the original unrelated donor. This is termed acquired tolerance.

Efforts to render homografts between genetically nonrelated partners successful have centered around treatment of the donor or recipient or both with various agents including radiation, radiomimetic drugs such as nitrogen mustard, and antimetabolites such as 6-mercaptopurine. To date these efforts have met with only limited success, and at the present time permanent survival of homografts in human beings is achieved only between identical twins. Thus the disciplines of immunology and genetics have become highly important to the surgeon striving to circumvent the homograft rejection barrier.

Technically successful transplantation of most major organs has already been accomplished, though in the absence of permanent survival of the homografts. For example, the lung, heart, liver, bowel, kidney, parathyroids and bone marrow have been transplanted with temporary survival in the nonrelated recipient, only to be rejected in a period of days. In human studies, conspicuous success has attended the transplantation of a kidney from one identical twin to his partner, but in the absence of such close genetic relationships homografted kidneys have been rejected.

It is concluded that while the nature of the homograft rejection phenomenon is now better understood than ever

before, one must concede that a final solution of this barrier to clinical transplantation of tissue or organs between nonrelated individuals is not in sight. Nevertheless, the challenge is a worthy one, the clinical rewards envisioned are great, and the barrier need not be looked upon as insuperable.

REFERENCES

(1) **Algire, G. H.**: J. Natl. Cancer Inst., **4**, 1, 1943. (2) **Algire, G. H.**, and **Legallais, F. Y.**: Ibid., **10**, 225, 1949. (3) **Algire, G. H., Weaver, J. M.**, and **Prehn, R. T.**: Ibid., **15**, 493, 1954.

(4) **Barnett, W. 0.**: To be published. (5) **Berman, E. F.**: Mt. Sinai Hosp. J., **9**, 97, 1960. (6) **Berrian, J. H.**, and **Brent, L.**: Ann. New York Acad. Sci., **73**, 654, 1958. (7) **Billingham, R. E., Brent, L.**, and **Medawar, P. B.**: Transp. Bull., **5**, 377, 1958. (8) **Billingham, R. E., Brent, L., Brown, J. B.**, and **Medawar, P. B.**: Ibid., 6, 410, 1959. (9) **Brent, L.**: Progr. in Allergy, **5**, 271, 1958. (10) **Brooks, J. R., Sturgis, F. H.**, and **Hill, G. J.**: Ann. New York Acad. Sci., **87**, 482, 1960.

(11) **Converse, J. M.**, and **Rogers, B. O.** (Consulting Editors): Fourth Tissue Homotransplantation Conference. Ibid., **87**, 1, 1960. (12) **Converse, J. M., Ballantyne, D. L.**, and **Woisky, J.**: Ibid., **73**, 693, 1958. (13) **Conway, H.**: Discussion of Sterling's paper. Ibid., **87**, 478, 1960. (14) **Crowell, J. W.**, and **Smith, E. E.**: Am. J. Physiol., **186**, 283, 1956.

(15) **Dempster, W. J.**: Brit. J. Surg., **40**, 447, 1953. (16) **Dunphy, J. E.**, and **Jacob, S. W.**: New England J. Med., **264**, 371, 1961.

(17) **Eichwald, E. J., Silmser, C. R.**, and **Wheeler, N.**: Ann. New York Acad. Sci., **64**, 737, 1957.

(18) **Goldstein, M.**, and **Baxter, H.**: Ibid., 73, 564, 1958.

(19) **Hardin, C. A.**, and **Kittle, C. F.**: Science, **119**, 97, 1954. (20) **Hasek, M., Hraba, T.**, and **Hort, J.**: Ann. New York Acad. Sci., **73**, 570, 1958. (21) **Hume, D. M., Jackson, B. T., Zukoski, C., Lee, H. M., Kauffman, H. M.**, and **Egdahl, R. H.**: Ann. Surg., **152**, 354, 1960. (22) **Hume, D. M., Merrill, J. P., Miller, B. F.**, and **Thorn, G. W.**: J. Clin. Invest., **34**, 327, 1955.

(23) **Jacob, S. W., Gowing, D.**, and **Dunphy, J. E.**: Am. J. Surg., **98**, 55, 1959.

(24) **Kelly, W. D., Lamb, D. L., Varco, R. L.**, and **Good, R. A.**: Ann. New York Acad. Sci., **87**, 187, 1960.

(25) **Lapchinsky, A. G.**: Ibid., **87**, 539, 1960. (26) **Lawrence, H. S.**: Ann. Rev. Med., **11**, 207, 1960. (27) Idem: Physiol. Rev., **39**, 811, 1959. (28) **Lillehei, R. C., Goott, B.**, and **Miller, F. A.**: Ann. Surg., **150**, 543, 1959.

(29) **Mann, F. C., Priestly, J. T., Markowitz, J.**, and **Yater, W. M.**: Arch. Surg., **26**, 219, 1933. (30) **Medawar, P. B.**: J. Anat., **79**, 157, 1945. (31) Idem: Nature, **182**, 62, 1958. (32) **Medawar, P. B.**, and **Russell, P. S.**: Immunology, **1**, 1, 1958. (33) **Meeker, W. R., Condie, R. M., Good, R. A.**, and **Varco, R. L.**: Ann. New York Acad. Sci., **87**, 203, 1960. (34) **Merrill, J. P.**: Physiol. Rev., **39**, 860, 1959. (35) **Merrill, J. P., Murray, J. E., Harrison, J. H., Friedman, E. A., Dealy, J. B., Jr.**, and **Dammin, G. J.**: New England J. Med., **262**, 1251, 1960. (36) **Moore, F. D., Wheeler, H. B., Demissianos, M. D., Smith, L. L., Balankura, O., Abel, K., Greenberg, J. B.**, and **Dammin, G. J.**: Ann. Surg., **152**, 374, 1960. (37) **Murray, J. F., Merrill, J. P.**, and **Harrison, J. H.**: Ibid., **148**, 343, 1958.

(38) **Nakic, B., Nakic, Z.**, and **Silobrcic, V.**: Nature, **186**, 322, 1960. (39) **Neptune, W. B., Cookson, B. A., Bailey, C. P., Appler, R.**, and **Rajowski, F.**: Arch. Surg., **66**, 174, 1953. (40) **Neptune, W. B., Weller, R.**, and **Bailey, C. P.**: J. Thor. Surg., **26**, 275, 1953.

(41) **Porter, K. A.**: Ann. New York Acad. Sci., **87**, 391, 1960. (42) **Porter, K. A., Moseley, R.**, and **Murray, J. E.**: Ibid., **73**, 819, 1958. (43) **Preston, F. W.**: Surg., Gynec. & Obst., **111**, 633, 1960.

(44) **Rogers, B. O.** (Consulting Editor): Third Tissue Homotransplantation Conference. Ann. New York Acad. Sci., **73**, 539, 1958.

26, 125, 1960. (46) **Simonsen, M.**: Acta path. et microbial. Scandinav., **32**,

(45) **Schubert, W. K., Fowler, R. J., Martin, L. W.**, and **West, C. D.**: Transp. Bull.,

36, 1953. (47) **Snyderman, R. K.:** Ann. New York Acad. Sci., **73**, 561, 1958. (48) **Sterling, J. A.:** Ibid., **87**, 476, 1960. (49) **Stetson, C. A.,** and **Jensen, E.:** Ibid., **87**, 249, 1960.
(50) **Toolan, H. W.:** Ibid., **73**, 546, 1958. (51) **Trentin, J. J.:** Ibid., **73**, 799, 1958.
(52) **Uphoff, D. E.:** J. Natl. Cancer Inst., **20**, 625, 1958.
(53) **Webb, W. R., Howard, H. S., Neely, W. A., Wax, S.,** and **Hagaman, D.:** J. Thor. Surg., **37**, 361, 1959. (54) **Woodruff, M. F. A.:** The Transplantation of Tissues and Organs. Springfield, Ill.: Charles C Thomas, 1960.

PROCEEDINGS OF

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Chorionic Gonadotrophin Activity in Euthyroid and Hypothyroid Ovaries. J. H. LEATHEM, PH.D. (Bureau of Biological Research, Rutgers University). Chorionic gonadotrophin will increase ovarian weight and stimulate the formation of lutein tissue in euthyroid animals. However, in hypothyroidism, this gonadotrophin will cause a more pronounced ovarian weight response and the formation of follicular cysts. Biochemical changes in ovarian composition associated with the two states have been studied. In euthyroidism, chorionic gonadotrophin increases ovarian total RNA and DNA, ascorbic acid, lactic acid and alkaline phosphatase. Concentration of RNA and DNA, ascorbic acid cholesterol, glycogen and alkaline phosphatase decrease. Uptake of P_{32} is enhanced and Na content of the tissue increases. The hypothyroid state failed to influence ovarian biochemistry including oxygen consumption and rate of glycolysis. However, in hypothyroidism, the gonadotrophin stimulus accentuated the biochemical changes observed in euthyroid ovaries. In addition, the formation of cystic ovaries was characterized by an increase in oxygen consumption, a reduced uptake of P_{32} and a decrease in ascorbic acid, free cholesterol and Na concentrations. Cessation of chorionic gonadotrophin is followed by spontaneous ovarian regression in euthyroidism but not in hypothyroidism. Furthermore, correction of the hypothyroid state in cases of established ovarian cysts did not correct the ovarian biochemistry even when the cysts had been surgically removed. The stimulus of a gonadotrophin during hypothyroidism appears to invoke a lasting biochemical change in the ovarian stroma.

Neurohumoral Regulation of Luteinizing Hormone (LH) Secretion. S. M. MCCANN, M.D., S. TALEISNIK, M.D., AND R. ABRAMS, D.D.S. (Department of Physiology, University of Pennsylvania School of Medicine). LH secretion was measured by ovarian ascorbic acid depletion in rats pre-treated with gonadotrophins. Mere insertion of a needle into the median eminence evoked LH release, whereas little or no effect was obtained on insertion at a variety of other hypothalamic loci. Positive results were also obtained from insertions into the lateral hypothalamus and amygdaloid nucleus. Lesions in the median eminence and in the suprachiasmatic region (the former inducing persistent diestrus and the latter constant vaginal estrus) prevented the rise in plasma LH which followed ovariectomy and were associated with subnormal values of hypophysial LH content. The rise in pituitary LH content which follows ovariectomy was blocked by median eminence lesions but not by those in the suprachiasmatic region. Acidic extracts of rat stalk-median eminence tissue evoked ascorbic acid depletion on injection into the assay animals.

Experiments with hypophysectomized assay rats indicated that at least part of this activity of the extracts was caused by LH secretion. The activity could not be accounted for by the content of known pharmacologically active agents in the extract, such as vasopressin and oxytocin. These stalk-median eminence extracts produced a significant elevation of the LH titer in plasma of ovariectomized rats pre-treated with estrogens. Little or no activity was found in extracts of cerebral cortex or other hypothalamic areas. It is suggested that an LH-releasing factor, secreted by the median eminence, regulates LH secretion by the adenohypophysis.

Activators and Inhibitors of Steroid Hydroxylation by Adrenocortical Microsomes. D. Y. COOPER, O. FOROFF AND OTTO ROSENTHAL (Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania). We have previously shown that 10 μM catecholamines and 10 μM ascorbate respectively accelerate and inhibit by about 50% 21 hydroxylation of steroids by adrenocortical microsomes from steer adrenals. We have now studied the action of enzyme inhibitors on this reaction. Microsomes, isolated in 0.25 M sucrose solution and washed in 0.15 M KCl were added to a 0.15 M NaCl-2% bovine albumin medium containing 20 mM glycylglycine buffer pH 7.4; 0.6 mM TPN; 3.3 mM glucose-6-phosphate; 0.4 units glucose-6-phosphate dehydrogenase and 0.2 mM 17αOH-progesterone. Steroid formation (2 hrs. 37° C. air), determined with the Silber-Porter reaction, averaged 300 μg. per mg. microsomal N.

Twenty-one hydroxylation was accelerated by O.1 mM cyanide (40 to 60%) or diethyldithiocarbamate (50 to 150%) which also prevented ascorbate inhibition. Inhibitory were 1 mM Versene (25%) or azide (30%); 0.1 mM$\alpha\alpha'$ dipyridyl (30%) and 0.01 mM o-phenanthroline (60%). Higher concentrations of the latter two inhibited completely.

It appears that two types of metal ions are operative in the microsomal system 1) activators (Cu?) of TPNH oxidation, directly or via ascorbate, preventing function of the coenzyme in hydroxylation and 2) activators (Fe^{2+}?) of the steroid hydroxylation. The nature and site of action of these cations are being studied.

BOOK REVIEWS AND NOTICES

LA SEXUALITÉ DES BACTÉRIES. BY E. L. WOLLMAN AND F. JACOB, L'Institute Pasteur. Pp. 248; 23 ills. Paris: Masson et Cie, 1959. Price, 3,000 fr.

THE authors have published much original research on bacteriophage and microbial genetics. Therefore, they are well qualified to prepare for French-reading scientists a monograph on sexuality in bacteria. Contained herein are the authors' own researches on lysogeny and genetic recombination in *E. coli* as well as an authoritative discussion of the pertinent world literature. Of particular interest to the writer is the section on sexual differentiation. This paperbound volume is a Pasteur Institute Monograph and it well deserves a place in that distinguished series.

E.S.

FUNCTIONAL NEURO-ANATOMY. BY A. R. BUCHANAN, M.D., University of Colorado School of Medicine, 4th ed. Pp. 377; 273 ills., 18 in color, Philadelphia: Lea & Febiger, 1961. Price, $8.50.

THIS text is an amplification of a syllabus for the author's class in neuroanatomy. A functional approach and discussion of clinical applications of neuroanatomy are emphasized. The restriction of the text to essential material and simplification of the illustrations that are semidiagrammatic line drawings will help to make this text very useful for the student.

E.S.

AN INTRODUCTION TO PHARMACOLOGY. BY J. J. LEWIS, Senior Lecturer in Experimental Pharmacology, University of Glasgow. Pp. 826; 164 ill., Edinburgh and London: E. & S. Livingstone Ltd., 1960 (The Williams & Wilkins Co., Baltimore, U.S. Agents). Price, $11.00.

THIS text is an elementary but good introduction to the subject of Pharmacology with relatively less emphasis on medical pharmacy and therapeutics than is found in the usual textbooks of Pharmacology for medical students. Relatively more emphasis is placed on general principles, some of which are illustrated with considerable clarity. Pertinent background material in physiology, biochemistry, microbiology and other subjects is given frequently as an introduction to a group of drugs, and is presented clearly yet concisely. Many of the illustrations are simple but extremely informative. Coverage is very broad from the standpoint of the number of drugs dealt with, but necessarily brief with respect to each group of drugs. Much of the material is presented in essentially outline form. Due respect is given to chemical formulae and structure-activity relationships. A good introduction but not a replacement for larger standard texts.

R.S.

SYNTHESIS AND ORGANISATION IN THE BACTERIAL CELL. BY ERNEST F. GALE, University of Cambridge, England: Pp. 110; 5 plates and 14 ills. New York: John Wiley & Sons, Inc., 1959. Price, $3.50.

THIS pocket-sized book contains three lectures delivered at Rutgers University in 1959 as the third of the series of Ciba Lectures in Microbial Biochemistry. In them Dr. Gale first relates cell structure to metabolic processes, and then concentrates upon microbial synthesis of nucleic acids and proteins. The subjects he discusses are of great importance to microbiologists and to biochemists particularly, but they have much broader significance, for the mechanisms of cell growth will not be understood until a much wider knowledge of nucleic acid-protein synthesis is achieved. Our state of knowledge in this area is clearly and expertly explained in these lectures.

Readers interested in the modes of action of chemotherapeutic agents will find that the author does not neglect his own extensive researches in this area. The discussions of this as well as the other subjects are sophisticated and, therefore, written at the level of the graduate student or specialist in microbial biochemistry.

E.S.

RECENT PROGRESS IN HORMONE RESEARCH, PROCEEDINGS OF THE LAURENTIAN HORMONE CONFERENCE, 1958. Edited by GREGORY PINCUS. Pp. 504; illustrated. New York: Academic Press, Inc., 1959. Price, $12.50.

THE annual Laurentian Hormone Conferences are always stimulating meetings. The program is planned to highlight important modern developments in hormone research rather than to emphasize a single subject.

This volume is divided into four sections. The first section consists of five papers on

the general subject of the Pituitary Hormones. The section on Aspects of Steroid Chemistry and Metabolism is made up of five papers. There are two papers devoted to Aldosterone Chemistry and Physiology. Of particular interest is the work by Gordon Farrell on factors influencing the secretion of aldosterone. The final section of four papers is under the general heading of Hormones and Metabolism. The discussions following each presentation are interesting and provocative. B.C.

RYPINS' MEDICAL LICENSURE EXAMINATIONS. Edited by WALTER L. BIERRING, M.D., Secretary-Editor, Federation of State Medical Boards of United States; Professor Emeritus, State University of Iowa. Pp. 805. Philadelphia: J. B. Lippincott Co., 1960. Price, $11.00.

THIS ninth edition of this popular review-for-examination book has been thoroughly revised and brought up-to-date. Fourteen outstanding authorities in various fields have contributed chapters dealing with their specialty, together with a series of essay questions with which the student can check his knowledge. The first chapter by Dr. Bierring discusses the general philosophy of examination, the various methods of posing questions, and sample questions from the Federation of State Medical Boards of the United States.

One of the great difficulties in the preparation of a book of this type is the need for excessive condensation of the material covered. The reviewer felt he could sense the strain that must have been involved for the authors as they attempted to reduce their subject to the required number of pages. It is of interest, for example, that the field of internal medicine, including skin, central nervous system, and mental disease, is given only 59 pages! Would it not be better to devote the entire book to questions, both of the essay and objective type, so that the student may be made aware of the weak spots in his education and be thus stimulated to refer to more exhaustive texts? T.D.

PROBLÈMES D'ULTRASTRUCTURES ET DE FONCTIONS NUCLÉAIRES. Edited by J. ANDRE THOMAS, The Sorbonne, Paris. Pp. 222; 67 ills. Paris: Masson et Cie, 1959. Price, 42 NF.

THIS paperbound monograph is the fifth of a series entitled "Biocytologica" produced under the direction of Prof. Thomas. The central theme is cellular, or more properly called in recent years, molecular, biology. Five investigators contributed 6 chapters to this volume, each author dealing with a subject to which he has personally contributed a substantial amount of new knowledge. For example, Baud discusses the structure of nuclear membranes as revealed by anistropic optics and he includes some beautiful photographs to support his points. On an equally high plane are contributions on RNA (Fica), DNA-proteins and chromosomes (Lenormant) and the cybernetics of cells (Glinos). Worthy of special commendation are Grasse's electron micrographs of chromosomal structures, and the extensive bibliography that follows Faure's chapter on nucleic acids.

Biologists, interested in the biochemistry of cells but not intimately acquainted with the original publications of these men, will find here much valuable information if they can read scientific French. E.S.

CLINICAL ORTHOPEDICS, NUMBER 17, SUMMER, 1960. Edited by ANTHONY F. DEPALMA, M.D., Professor of Orthopedics, Jefferson Medical College, Philadelphia. Pp. 387; 204 ills. Philadelphia and Montreal: J. B. Lippincott Company, 1960. Price, $7.50.

THIS volume contains few clinical data but abounds in clinical physiology and pathology of bone. The following titles of some of the articles attest to this: Additional Abbreviations on Bone Crystal Dimensions, the Ivory Core of Tusks and Teeth, Isotope Study of Bone Salts, Crystal-Collagen-Water Relationships in Bone Matrix, and many others. As a text it indicates research is active in various facets of Orthopedics. The book should be of great interest to the physiologist, pathologist and research worker. It is not recommended for the medical student, nurse or physiotherapist. The Editor is to be congratulated on the varied articles included in the text; the bibliography for each is more than adequate. A.S.

SELECTIVE TOXICITY. BY ADRIEN ALBERT, D.SC. (LOND.), PH.D. (MED.), Professor of Medical Chemistry, Australian National University. 2nd ed. Pp. 233; 36 ills. New York: John Wiley & Sons, Inc., London: Methuen & Co., Ltd., 1960. Price, $5.50.

THIS book is directed primarily at post-graduate readers interested in Pharmacology and Toxicology. It is about drugs or chemical agents which selectively injure some kinds

of cells and not others, with particular attention to the physical and chemical mechanisms involved. Part one (four chapters) is devoted to topics of general interest and includes such topics as receptors, permeability of membranes, drug resistance, the value of measurement, and others. The second part (9 chapters) is more sophisticated and aimed at more advanced students; it concerns the relationship between structure and biological activity, using selected groups of compounds as examples. Emphasis is placed on physicochemical mechanisms. A good, but not simple, introduction to the problems of general or fundamental Pharmacology for the advanced and interested student. R.S.

CYTOLOGIE DER CEREBROSPINALFLUESSIGKEIT. BY DOZENT DR. MED. HABIL. JOHANNES SAYK, Neurologische Abteilung der Psychiatrischen Klinik der Friedrich-Schiller-Universitaet Jena. Pp. 175; 90 ills. Jena: Gustav Fischer, 1960. Price, DM 44.40 ($10.00).

THE first half of this excellent book gives a full description of the various methods used to obtain, to count, and to stain the cells of the cerebrospinal fluid; and a review of the cytochemistry, the origin, and the function of these cells. The author recommends sedimentation of the cells in a specially constructed chamber, the sedimentation being propelled by the capillary attraction of filter paper. This ideal technique permits perfect conservation and staining of the cells. The second half of the book contains a comprehensive description of the liquor cytology in the inflammatory diseases, multiple sclerosis, hemorrhage, tumors, lues and arteriosclerosis of the central nervous system. This is correlated with the chemistry of the liquor. The illustrations are excellent. Many are in color. The book is highly recommended to all who are interested in the cytology of body fluids; it is a must for the students of liquor cytology. W.E.

DIE WIRBELSAEULE IN FORSCHUNG UND PRAXIS. Edited by PROF. DR. MED. H. JUNGHANNS, Oldenburg. Referatenband I. 1960 (Volume 16). Edited by DR. MED. F. BIEDERMANN. Pp. 196. Stuttgart: Hippokrates-Verlag, 1960. Price, DM 19.80.

THIS is Volume 16 of a series of books entitled "The Vertebral Column in Research and Practice." Like volumes 4, 6, 8, 10 and 12 of this series, the present book contains individual reviews of papers and books dealing with the vertebral column (1151 papers and 56 books were reviewed in these 5 volumes) and a collective review by H. Jesserer on osteoporosis, rickets and osteomalacia, osteitis fibrosa generalisata, and osteosclerosis. As in previous volumes, one-sided printing and the international decimal classification were used in order to facilitate filing of the reviews. The volume should be of considerable usefulness to physicians who are interested in the spine. W.E.

DIE WIRBELSAEULE IN FORSCHUNG UND PRAXIS. Edited by PROF. DR. MED. H. JUNGHANNS, Oldenburg. DIE SPONDYLOSIS CERVICALIS. VOL. 17. BY M. AUFDERMAUR. Pp. 79; 53 ills. Stuttgart: Hippokrates-Verlag, 1960. Price, DM 16.80.

ONE hundred cervical spines (from the 2nd cervical to the 1st thoracic vertebra) were collected at random, they were x-rayed, and one half of each spine was macerated, the other studied histologically. In cases of kyphosis of the lower cervical vertebra, the upper vertebra revealed hyperlordosis in all cases. Spondylosis cervicalis was very common, but while arthrosis increased with age, spondylosis declined after the 7th to 8th decade. Spondylosis deformans is initiated by tearing of discs which may undergo fibrosis or ossification. Prolapse of the nucleus pulposus occurs chiefly in the lower cervical spine. There was no correlation between even considerable anatomical changes and clinical symptomology. The book is well illustrated. The photographs are excellent. Both orthopedists and roentgenologists should profit from a study of this interesting paper. W.E.

DELINQUENT AND NEUROTIC CHILDREN. BY IVY BENNETT, PH.D. Pp. 531. New York: Basic Books, Inc., 1960. Price, $10.00.

IVY BENNETT, a British psychologist and psychoanalyst, attempts in this worthwhile book to describe the differences in the personality and background of delinquent children as contrasted to neurotic children. Her work is the result of a project suggested by her late mentor, Kate Friedlander, systematically to compile and analyze clinical material to test aspects of psychoanalytic theory about the development of neurotic and delinquent children. A careful selection and summarization of 50 cases of delinquent children and adolescents evaluated and treated in the English Rural Child Guidance Service are

presented and contrasted with 50 comparable cases of neurotics.

Dr. Bennett comprehensively reviewed the literature on delinquency and correlates it with her own findings. The study substantiates psychoanalytic thinking about delinquent children; namely, that the problem of most delinquent children is related to faulty superego formation due to factors as absence or early interruptions in a secure mother-child relationship, broken homes, poor parental figures to identify with and grossly traumatic or inconsistent discipline. W.C.

Praktikum der Immunhaematologie. By Prof. Dr. H. H. Hennemann, Medizinische Universitäts-Poliklinik Koeln. 2nd ed. Pp. 148; 33 ills. Stuttgart: Georg Thieme Verlag, 1960 (Intercontinental Medical Book Corp., New York, U.S. Agents). Price, DM 19.80 ($4.70).

This manual was designed to guide physicians and medical technologists in the study of immunohematological problems in hospital patients. It contains a description of various methods used in the study of cold agglutinins and other antierythrocytic autoantibodies (including the Coombs test); of special methods used in acquired hemolytic anemias caused by antibodies (including tests for sedimentation rate, erythrophagocytosis, and mechanical and osmotic resistance of erythrocytes); of methods used to demonstrate antithrombocytic and antileukocytic serum factors (including the L.E. test); of methods to study heterophil antibodies, to determine complement and properdin, and to measure the hydrogen-ion concentration; and of other methods. The usual serological methods and tests for blood groups are not included. Each chapter is introduced by a brief characterization of the problem and its clinical significance. The employment of this useful booklet requires knowledge of the German language. W.E.

Respiration, Physiologic Principles and Their Clinical Applications. By P. H. Rossier, A. A. Bühlmann, and K. Wiesinger. Translated from the German Edition by P. C. Luchsinger and K. M. Moser. First English Edition. Pp. 404; 95 ills. St. Louis: C. V. Mosby Co., 1960. Price, $15.75.

This translation makes available a valuable work in the field of respiration. The work is authoritative and has provided a somewhat different approach to some of the problems considered. The illustrations are well chosen. It is helpful to have physiology, methods and pathology combined in one book. The appendices are useful and the bibliography extensive. Students and researchers in respiration will find this book useful. M.O.

Atlas of Bacterial Flagellation. By Einar Leifson, Stritch School of Medicine. Pp. 171; 69 ills. and 14 tables. New York: Academic Press, Inc., 1960. Price, $7.00.

For many years Leifson has made an intensive study of bacterial flagella. He devised better ways to visualize them. And he became convinced that their shape and arrangement in the cell have much taxonomic value. His thesis is supported by many excellent photomicrographs, all made by him from his own stained preparations. Because each figure contains 5 to 30 separate photographs this volume is richly supplied with them (to the point of being somewhat repetitious). Little text is needed since his photographs portray clearly the author's messages, for example, the fact that the possession of flagella by group D streptococci may constitute a means of identification overlooked by bacteriologists up to now. E.S.

Bleeding Syndromes. By Oscar D. Ratnoff, M.D., Associate Professor of Medicine, Western Reserve University School of Medicine. Pp. 287; illustrated. Springfield, Ill.: Charles C Thomas, 1960. Price, $8.50.

One of the most difficult fields of medicine for understanding among the various clinical disciplines is that associated with problems in hemostasis. Many of the previous books published in this area are written for the specialist whose training in laboratory procedures allows him to understand the complexities of the nomenclature and tests involved. The author has written an easy reading compilation of useful practical information about hemorrhagic diseases in which he stresses the clinical approach to these patients which would be of value to any physician concerned with the day-to-day care of patients with bleeding syndromes. In doing this, Dr. Ratnoff has drawn upon his own vast experience in the handling of these patients plus an extensive, well chosen bibliography.

Following a chapter on the physiology of hemostasis, the chapter on the importance of the history, physical examination and significance of the commonly used laboratory tests

sets the tone of the book, with its emphasis on the clinical problems involved.

This book is to be recommended to all physicians who have the responsibility for the management of these patients. J.D.

AN INTRODUCTION TO FUNCTIONAL HISTOLOGY. BY GEOFFREY H. BOURNE, M.Sc., D.Sc., D.Phil., Professor and Chairman, Department of Anatomy, Emory University, Atlanta. 2nd ed. Pp. 263; 181 ills. Boston: Little, Brown and Co., 1960. Price, $8.00.

THIS book offers to undergraduates and graduates alike an exciting introduction to the study of histo- and cytologic structure and function. In many instances, basic concepts in such related fields of histochemistry, electron microscopy and cell physiology are emphasized in both text and illustrative material. Written in a fashion which can maintain the interest of the novice, the various chapters comprise enjoyable reading. The selection and use of certain photographs, line drawings and charts have much value in the presentation of the subject matter; although a few of these could be of a finer technical reproduction (charts) while certain others (nerve electron micrographs) might represent those of more recent vintage. C.R.

CLINICOPATHOLOGICAL CONFERENCES OF THE MASSACHUTTS GENERAL HOSPITAL: SELECTED MEDICAL CASES. BY BENJAMIN CASTLEMAN, M.D., AND H. ROBERT DUDLEY, JR., M.D., Chief, James Homer Wright Pathology Laboratories, Massachusetts General Hospital and Clinical Professor of Pathology, Harvard Medical School and Assistant in Pathology, Massachusetts General Hospital and Instructor in Pathology, Harvard Medical School, respectively. Pp. 289; 152 ills. Boston and Toronto: Little, Brown and Company, 1960. Price, $12.50.

THIS book contains 50 cases presented as the weekly Clinicopathological Conferences published in the New England Journal of Medicine as the Case Records of the Massachusetts General Hospital. As soon as one mentions the CPC from the Massachusetts General Hospital and Harvard Medical School little more needs to be said. The 50 cases were selected for their wide coverage of the field of medicine, for the varied discussions prompted by the particular case and to demonstrate current laboratory aids in confirming a diagnosis. Some of the cases that were selected were done so for historical reasons; these earlier cases are followed by addenda which indicate in the majority of instances that the discussors arrived at the correct diagnoses but that their task would have been made easier by current medical diagnostic aids. In the preface the authors point out the value and popularity of the CPC and indicate that it is not a "guessing game" but an exercise demonstrating the mental gymnastics that the discussor goes through in arriving at a diagnosis. This is an excellent book and contains many "pearls" along with key references. The authors are to be commended for their selection of cases and for their presentations including pertinent illustrations. A.P.

PSEUDOMONAS AERUGINOSA INFECTIONS. BY CLAUDE E. FORKNER, JR., M.D., Cornell University Medical College. Pp. 104; 23 ills. New York: Grune & Stratton, Inc., 1960. Price, $5.25.

THIS small volume belongs to the Modern Medical Monographs series. In it the author has collected references (465 in all) relating to the clinical manifestations and therapy of *Pseudomonas aeruginosa* infections. But he devotes less than 5 pages to the organism itself, and he says nothing about its laboratory identification. Furthermore, reference to the relatively high resistance of *Ps. aeruginosa* to disinfectants is limited to a short paragraph in the summary.

The incidence of pseudomonas infections has increased in recent years and it will probably continue to do so. Consequently there is a need for the information Dr. Forkner has assembled. E.S.

A CLINICAL PROSPECT OF THE CANCER PROBLEM. BY D. W. SMITHERS, M.D., F.R.C.P., F.F.R., Professor of Radiotherapy in the University of London. Pp. 232; 42 ills. Edinburgh and London: E. & S. Livingstone Ltd., 1960. Price, $8.50.

THIS remarkable book serves as an introduction to a series of monographs on neoplasms at individual sites. If the volumes to follow approximate this one in quality, they should be on the shelves of every doctor who comes in contact with malignant disease.

Although the author is intimately concerned with the detailed care of patients with cancer, he takes a broad philosophical approach to the problem. His principal thesis is that cancer is not a disease entity, and that the cancer cell does not have some peculiar added malignant quality.

The author deplores ultra-specialization in the field and points out that there are too few doctors who take a wide view of the problem. He makes a plea for lectures on neoplasia, as a whole, in the medical curriculum.

Although the view is panoramic, there are many direct references to clinical and basic science problems. The book is highly recommended and should prove exciting reading for clinician and research worker alike. N.L.

MICROBIOLOGY: HISTORICAL CONTRIBUTIONS FROM 1776 TO 1908. Edited by RAYMOND N. DOETSCH. Pp. 233; 33 ills. New Brunswick, N.J.: Rutgers University Press, 1960. Price, $5.00.

SEVENTEEN papers by fifteen pioneers in bacteriology are reproduced, some translated into English for the first time. Each paper is preceded by an introduction in which the editor explains why he selected it. And each one describes either a major contribution to technology (Koch, Beijerich, Winogradsky, and Ehrlich) or a breakthrough to new concepts (Spallanzani, Pasteur, Lister, Warington, and Erwin Smith). As the editor points out, the choice of papers for inclusion in an historical anthology is bound to be influenced by his personal interests. This reviewer has no reason to disagree with Doetsch's selections; rather he suggests that a similar volume tracing the development of medical microbiology would be equally worth while. E.S.

PROGRESS IN ENDOCRINOLOGY. PART I. NEUROENDOCRINOLOGY AND ENDOCRINOLOGY OF THE THYROID AND PARATHYROID GLANDS. Edited by K. FOTHERBY, J. A. LORAINE, J. A. STRONG AND P. ECKSTEIN. Memoirs of the Society for Endocrinology No. 9. Pp. 166; 50 ills. New York: Cambridge University Press, 1960. Price, $8.50.

THE proceedings of the 1959 joint meeting of the British Society for Endocrinology and societies representing the Acta Endocrinologica Congresses, is an excellent addition to the series of endocrinological memoirs so well known throughout the world. Selected topics relating to those matters described in the title are well presented by contributors from many nations. Within the recognized limitations of such a text, the subjects are representations of current work in each field. The text is elucidated by diagrams, figures and tables. The usual provocative discussions follow each monograph and the bibliography is ample. A.B.

CELL PHYSIOLOGY OF NEOPLASIA. Edited by the STAFF OF THE UNIVERSITY OF TEXAS M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE. Pp. 653; illustrated. Austin: University of Texas Press, 1960. Price, $10.50.

THIS book is a collection of papers presented at the 14th annual symposium on cancer research held at the M. D. Anderson Hospital and Tumor Institute in 1960. There are 19 papers distributed among the divisions of 1) Nucleus, 2) Cytoplasm, 3) Nucleic Acids, and 4) Cell Growth and Development.

Each paper is well done, and well illustrated, by experts in their respective fields. Lively discussion follows each paper. The title of the book is somewhat misleading because much of the contents pertains, as it should, to physiology, morphology and dynamics of normal plant and animal cells as well as to neoplastic cells. Each paper bears a well documented review of recent literature in its area. Some are concerned almost entirely with elegant methodology, while others define results of recent work. Much of the experimental work is presented as pure, basic material without an attempt to relate the findings to oncology.

Histochemists, cellular physiologists, electron microscopists and oncologists should find the book of value.

The quality of the printing is marred only by frequent broken letters, "i" in particular. It is unfortunate that illustrations could not follow the order of the text better. The cost of the text seems reasonable. K.S.

THE GREAT EPIDEMIC. BY A. A. HOEHLING. Pp. 217; 13 illustrations. Boston and Toronto: Little, Brown and Company, 1961. Price, $3.95.

WITH the touch of a professional writer, Mr. Hoehling has produced in this monograph a moving, dramatic account of the influenza pandemic of 1918-19. Using many quoted statements of both lay and professional people, derived from public and official records, he follows the actions and reactions during the months of this great pandemic. His account describes the morbidity and mortality, in terms other than simple statistics, in army camps, aboard ships, in large cities as well as small towns, and in small islands—throughout the world. With sparkling style,

the urgency and severity of the situation is imparted to the reader.

Probably intended for lay, popular consumption, the book has been written with good technical advice. Physicians who did not experience the great pandemic would be rewarded by spending an evening with this monograph. K.S.

THE MANAGEMENT OF PEDIATRIC PRACTICE. BY HUGH C. THOMPSON, M.D. AND JOSEPH B. SEAGLE, M.D., Tucson Clinic. Pp. 172; 15 ills., 17 tables. Springfield, Ill.: Charles C Thomas, 1961. Price, $7.50.

THIS volume, bearing the subtitle "A Philosophy and Guide" is an inspiring expression of devotion to good medical practice among children. It is necessarily biased and the sentiments often represent a single approach to broad problems. Some issues, such as the role of nurse employees in practice, would meet with varied opinions elsewhere. In truth, this is a cursory recitation of recommendations primarily based on experiences from a well known clinic. It is doubtful whether the aspiring pediatric practitioner would learn very much by its perusal. Many more practical details awaiting the judgment of authors, such as these, are anticipated in the reading but are paraded in brief, if at all.

A.B.

Thermodynamics and Mechanics of Polymer Systems. BY F. R. EIRICH, B. D. COLEMAN, E. W. FISCHER, M. FIXMAN, M. KURATA, J. MAZUR, W. NOLL, A. PETERLIN, F. T. WALL, AND B. H. ZIMM. (Ann. New York Acad. Sci., Vol. 89, Art. 4). Pp. 141; illustrated. New York: New York Academy of Sciences 1961.

Report of Second African Conference on Bilharziasis. World Health Organization Technical Report Series No. 204. Pp. 37. Geneva: World Health Organization, 1960. Price, $.30.

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DR. RICHARD A. KERN, Temple University Hospital. Philadelphia 40, Pa.

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ILLUSTRATIONS accompanying articles should be numbered and have typed captions bearing corresponding numbers. For identification they should also have the author's name written on the margin or back. Line drawings and charts should be prepared with black India ink. Special arrangements can be made for printing color plates, elaborate tables or an excessive number of illustrations.

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